STELAR STRUCTURE IN THE DICOTYLEDONS.

A Resurvey based on

Considerations of Development.

by

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I declare that the work and composition of this thesis, "Stelar Structure in the Dicotyledons", is entirely my own.

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GENERAL INTRODUCTION.

The origin and evolution of the angiosperm stele, especially the dicotyledon stele, has been the subject of much investigation and discussion. In the main the arguments have been drawn from comparative anatomy and palaeobotany, although seedling anatomy was much stressed at one period. Great weight has been laid on palaeobotanical evidence, especially in seeking for ancestral types from which to derive the angiosperms. This is seen to be natural when the sequence of discovery of the important fossil types is remembered. With the discovery of the 'Pteridosperms', the Bennettitales and Cycadofilicales a great impetus was given to speculation on the origin of the angiosperms. Jeffrey's (17) theories of the origin of the siphonostele from the protostele (based on fossil material) and Tansley's (37) studies of stelar development in the ferns, suggested a connexion of the angiosperm stele with these earlier groups. (See also Coulter and Chamberlain (9)).

Further work on the stem anatomy of angiosperms was linked up with the problem of the evolution and significance of the herbaceous types and with the correlation of anatomy with systematy. The herbaceous type was considered to be derivative. The argument for this was two-fold, appeal being made to palaeobotany and also to comparative anatomy of existent forms.

The argument from palaeobotany was based on the well-known fact that in phyla other than the angiosperms extinct members reached a much greater magnitude of vegetative development than present-day representatives. (Vide especially the Lepidodendrid-Lycopod and Calamite-Equisetum series). This was reinforced by the current systematy which considered the wind-pollinated, apetalous tree forms as primitive. The present tendency to regard these as reduced and to redistribute them among the petaloid forms has robbed this argument of much of its value in the anatomical field.

The argument from comparative anatomy was largely based on the theory of recapitulation, although some writers emphasised this more than others. E.C.Jeffrey is the main protagonist of this theory In many papers and in his book "Anatomy of Woody Plants " (17) he stresses this view-point. For example (ibid., p. 191) he mentions the existence in Helianthus of a 'circular and complete woody cylinder' in the lower region of the epicotyl and multifasciculate structure in the upper region of the same plant as 'developmental evidence' pointing to the 'derivation of herbaceous forms from woody ones'. In Helianthus hirsutus he considers the 'outstanding' (i.e projecting from the stelar ring) foliar traces in the upper regions ' a condition very commonly found in extreme herbs which have very largely lost their woody structure.'

Thoday (38) disposes of this by showing that the foliar strands are 'outstanding' from the rest of the stele because of the long decurrence of the leaf-bases, this being seen even in the first node of the epicotyl: their subsequent 'depression' being due to greater cambial activity in the joining segments.

Sinnott and Bailey (31) say 'The base of most herbaceous stems is much stouter than the upper portion and often shows a close resemblance to a woody twig. On passing upwards from such a base to the more delicate portions of the stem we can readily observe the progressive decrease in cambial activity and increase in parenchymatous tissue which have caused the development of the herbaceous type.' (The italics are the present writer's: the passage quoted clearly shows the point of view of the authors.)

Thoday (38) shows that in Helianthus there is no increase in the proportion of parenchyma from the base upwards, only a change of scale in the whole stem, reflecting the increased vigour of primary growth. There is no difference in the ontogeny of

upper and lower internodes; the plan and sequence of development is the same, so that difference in detail cannot be given fundamental morphological significance.

The organisation of the node and in particular the significance of the 'foliar ray' has been extensively studied, notably by Jeffrey (17), Sinnott (30), and Sinnott and Bailey (32). The latter authors in "The Significance of the 'Foliar Ray' in the Evolution of Herbaceous Angiosperms" show that a continuous woody ring is not confined to the arboreal types nor a many-bundled stele to the herbaceous. They cite as examples of 'continuous' steles the following:- Liquidambar(tree), Lonicera (vine), Digitalis and Campanula (herbaceous stems). As examples of 'dissected' steles they give the following:- Platanus(tree), Dillenia (tree),Clematis (vine), Artemisia, Eupatorium (herbs). Inspection of their illustrations shows that they are dealing with mature stems, in which most of the xylem is secondary. The structures they refer to as 'foliar rays' are really the gap-residues, that is the remaining parenchymatous regions between the leaf-trace and the main stele: their organisation as 'rays' is secondary. This is fully discussed below, p. . They say that 'the herbaceous stem in general is essentially similar to the first year's growth of its woody prototype, differing mainly in the possession of a relatively thinner woody ring.'

Sinnott (30) examined the nodal structure throughout the range of dicotyledon families. He found that in general the number of leaf-traces and therefor of gaps was consistent within a family. He considered that the trilacunar gap (which is the most frequent) was the most ancient type, and that the unilacunar and multilacunar types were derived from it. The unilacunar is almost as frequent as the trilacunar gap, while the multilacunar occurs in very few families. The writer has examined the distribution

of these gap-types in connexion with the general survey of stelar patterns given below: some correlations are suggested.(See p. 43).

In the opinion of the writer further progress cannot be made without an extensive and intensive study of a). the nature and distribution of stelar patterns in the dicotyledons, b). the ontogeny of the stele as seen in the shoot apex of the established plant.

The anatomy of the seedling is not in itself a reliable guide to stelar evolution, because at this phase of the plant's life the problems of growth and adjustment are over-riding. Speedy establishment is the paramount necessity and morphological and anatomical compromises are to be expected. Only in the normally growing shoot apex can the true stelar plan of the plant show itself. For this reason the two aspects of the problem, investigation of adult structure and of development, must be undertaken concurrently. This has been done by the writer for a range of types covering all sections of the dicotyledons, and the following general considerations are offered, the supporting evidence being given below.

I). All normal dicotyledon steles are composed of foliar segments, delimited by parenchymatisation at the time of formation of the 'soubassements foliaires.'

2). All dicotyledon steles are dissected by leaf-gaps: therefore the old terminology of 'continuous' and 'dissected' steles cannot be maintained.(Alternative terminology based on considerations of development is proposed below).

3). All dicotyledon steles contain interfascicular parenchyma segments which are gap-residues: some types also contain interfascicular parenchyma of different origin(see description of multisect type below): in addition all steles contain a certain proportion of intrafascicular parenchyma.

ANALYSIS OF STELAR PATTERNS.

The wide range of variation seen in mature dicotyledon stem structure may be analysed under three headings:-

A).General plan of stele. B).Differentiation. C).Maturation.

A). General plan of stele.

The most important variation is in the proportion of size of the vascular segments to the gap-residues, which follows from variation in the relative sizes of trace, gap and main stele and is determined at the very initiation of the procambial stage by parenchymatisation of pith, cortex and gap. Whether the leaf trace developes as a single unit or is broken up into separate strands before vascular differentiation depends upon further radial parenchymatisation : this is the condition which it is proposed to call multisect and is described below.

B). Differentiation.

There are two distinct methods of differentiation of xylem from procambium.

i). Xylem elements differentiate in regular files alternating with files of parenchyma.

ii). Xylem elements differentiate irregularly and appear mixed with parenchyma in the maturing strand.

The amount of strictly primary xylem depends upon the time of initiation of cambium. If xylem differentiation precedes that of cambium, then some xylem will be primary: if cambium appears first, then all the xylem will be secondary. It will be seen that case i). above may arise in two ways, but that case ii). can only arise if xylem precedes cambium.

C). Maturation.

Fundamental differences in maturation and consequently in adult structure depend upon the activity of the interfascicular

cambium.

i). The gap-residues are maintained as parenchyma segments (which may be organised as 'rays'), resulting in an open stele.

ii). The gap-residues are closed by joining segments of vascular or non-vascular tissue, which is not as a whole organised as 'rays', resulting in a bound stele.

These differences outlined above appear to the writer to be of fundamental importance, much more so than detailed differences in the composition of secondary xylem and phloem. They may be used as criteria to distinguish the basic types of stelar structure found in the dicotyledons. It was found that normal dicotyledon stems fall into a comparatively small number of groups. Moreover by the use of a small number of symbols it is possible to write a short descriptive formula which embodies information under some or all of the above headings. (See next section). There are of course some stems which cannot be classified under the above scheme. For example some of the well-known aquatics with highly reduced centralised steles: they are so clearly habitat modifications and adaptations to physiological needs that they must be considered as outside the main body of the dicotyledons in structure. The same may be said of such highly parasitic forms as Rafflesia etc. What are ordinarily called 'anomalous' stems (e.g. possessing cauline bundles, abnormal secondary thickening etc.) can usually be interpreted in terms of the normal, so that they do not affect the general considerations given above. These will now be discussed more fully.

A). General plan of stele.

In addition to the variation in relative sizes of trace gap and main stele upon which depends the degree of dispersion of the vascular segments in the stem, there is also the question of whether the leaf trace developes as a single unit or not. In tracing the development of the leaf trace from the procambial stage two conditions are found:-

I). The leaf trace segment differentiates as a single unit.

II). The leaf trace segment is broken up into a number of small separate procambial strands by radial parenchymatisation before vascular differentiation begins. This condition does not appear to have been appreciated before. It is proposed to call the type of stele which results from this MULTISECT.

Within these two classes two further divisions can be made, depending upon the size of the leaf trace in proportion to its gap. In an endeavour to arrive at a quantitative definition, outlines of several hundred stems were made by means of a micro-projector, and clinometer measurements taken of the proportions of vascular segments and gap-residues. As a result, the following definitions can be suggested:-

a). The leaf trace fits its gap closely, so that the gap-residues are small, forming from 5-20% of the stelar ring in the internode. It is proposed to call this type of stele CONSOLIDATED.

b). The leaf trace is small in proportion to its gap, the gap-residues are large, forming 30-70% of the stelar ring. It is proposed to call this type of stele DISPERSED.

In all the multisect types examined the leaf traces were large, so that the terms consolidated and dispersed must be taken as applying to steles where the leaf trace differentiates as a unit.

Types of consolidated and dispersed steles are illustrated in Figures I,3,4,5 and I0-I7.

The following figures are given as examples of the proportions found in some consolidated and dispersed steles. (G.R.P. means gap-residue parenchyma).

CONSOLIDATED STELES.

G.R.P. < 5%		G.R.P. 10-20%	
Tilia	4%	Magnolia	10%
Populus	5%	Liriodendron	10%
Salix	5%	Corylus	11%
Betula	6%	Quercus	12%
		Garrya	12%
		Ulmus	12%
		Santalum	18%
		Carpinus	18%

DISPERSED STELES.

G.R.P. 30-40%		G.R.P. 40-50%		G.R.P. > 60%	
Ochna	30%	Physalis	40%	Clematis	60%
Dillenia	33%	Platanus	43%	Fumaria	66%
Hibbertia	35%	Moringa	49%	Lamium	68%
Datura	36%	Acer	51%		

MULTISECT STELES.

The multisect condition is particularly well seen in the Capparids. The leaf trace is large and while still in the procambial stage is seen to be a single arc, delimited in the usual way by the dorsal and ventral parenchymatisation of the soubassement foliaire. Before any differentiation of vascular elements has taken place however, radial parenchymatisation takes place across the segment in such a way as to isolate very narrow individual strips of procambium. The separating strips of parenchyma are as wide as or wider than the procambial groups and are largely vacuolated at the time of first differentiation of vascular elements.

Differentiation of xylem follows the regular type (see below) and in most cases the whole of the inner portion of the procambial strand becomes xylem. Additional intrasegmental parenchyma does not appear until after the establishment of the cambium.

It is difficult to know exactly how to describe these parenchyma segments. In the sense that they develope from procambium

after its original general delimitation, they might be called stelar, but they are n ot homologous with the intrasegmental parenchyma in such a stele as Tilia because the latter differentiates concurrently with the xylem while the former precedes it. It will be seen that the internode of Capparis and other multisect types contains interfascicular parenchyma of two kinds:-

i). gap-residue parenchyma, which can usually be distinguished by its position and greater width,

ii). parenchyma of the origin described above.

The distinction of origin of these two types of parenchyma does not appear to have been recorded before.

The secondary development of the multisect types vary. In Capparis where the vascular segments are extremely narrow and the parenchyma segments relatively wide, the stele is usually bound by the interfascicular cambium. (See Fig.28). In Fraxinus and other Oleaceae (Figs. 8-9) the stele remains open. In the less obvious examples it is easy to mistake the mature stele for the consolidated regular pattern, but studies of the differentiation make it clear. A study of the conspectus of Families showing the distribution of stelar patterns will show that the multisect condition is by no means rare. Its relation to the consolidated stele will be discussed below.

B). DIFFERENTIATION OF XYLEM FROM PROCAMBIUM.

There appear to be two distinct trends in the differentiation of vascular segments, associated with differences in stelar plan and with the organisation of the procambium itself.

I). Regular. The procambium differentiates into regularly alternating files of xylem and parenchyma, enlargement of the parenchyma cells beginning almost simultaneously with the appearance of the first protoxylem vessel. The first protoxylem elements to appear

are not in radial seriation but are in contact with each other.

This type of differentiation is associated with a consolidated stele. Only a small amount of flanking parenchyma intervenes between the arc of procambium and the main stele, so that it may be thought of as differentiating within a comparatively rigid framework. The pressure of the vacuolating cells of the subtending (dorsl and ventral) parenchyma have already compfessed the procambium into its characteristic shape. It is suggested that the additional tangential pressure exerted by the gap-residue and the intrasegmental parenchyma is responsible for the further moulding of the plastic procambial mass into a more regular mosaic than before, thus producing an appearance of radial seriation. This is borne out by comparison of serial sections which clearly show that several xylem elements in addition to the first are developed direct from procambium and must therefor be regarded as strictly primary. (See Figs. 48,49, 57, 65, 66.)

Further development proceeds as follows. Periclinal divisions indicating the inception of the vascular cambium first appear opposite the xylem files and several periclinal divisions take place in these cells before any arise across the files of parenchyma. At this stage the parenchyma cells are considerably but not fully vacuolated. They are elongated radially and the cells further from the pith have usually undergone radial division before the cambium is established, so that for the rest of its course the parenchyma is at least two cells wide. (Figs. 47,48.) Once established the vascular cambium produces both xylem and parenchyma in regular radial seriation. Examples of the type of vascular strand resulting from this kind of differentiation are shown in Figs. 22, 23, 26, 27, 28.

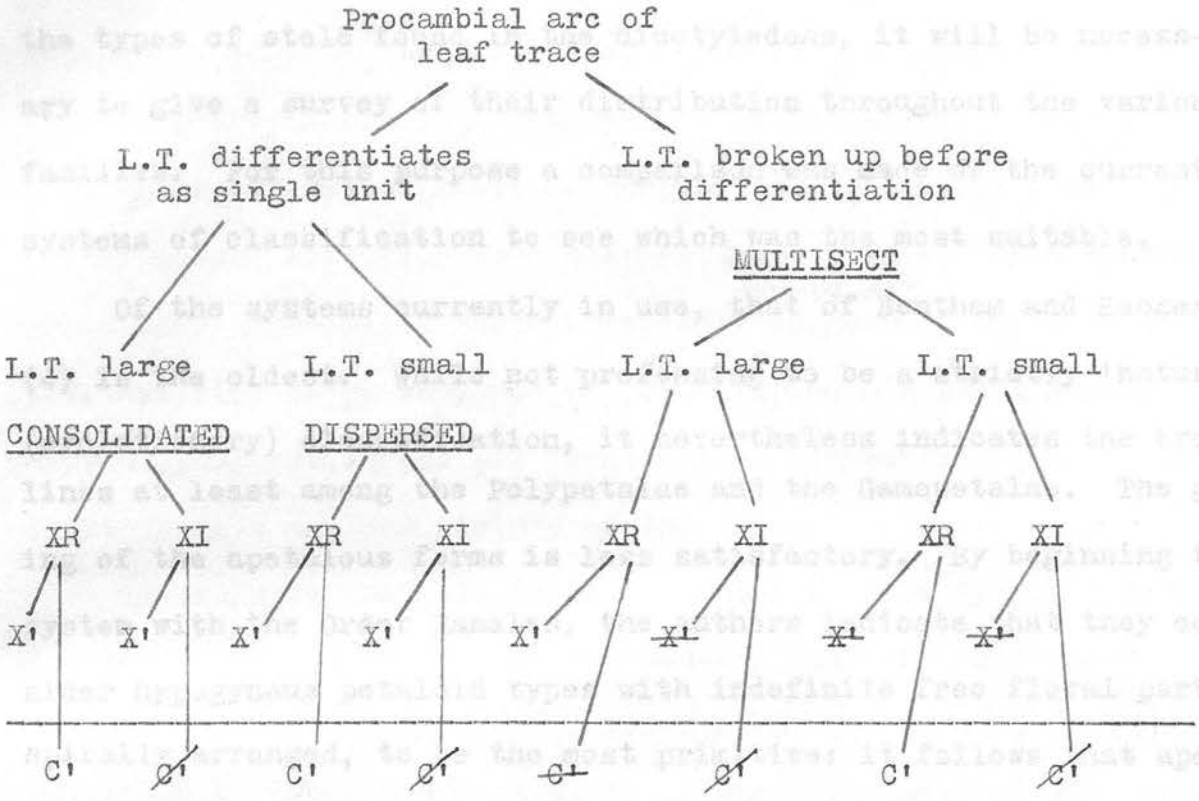
Regular xylem may arise in another way, namely from the activity of a precocious cambium: that is, regular periclinal divisions begin in the procambium before any differentiation of vascular elements. In these cases there is no primary xylem - all is secondary. This type of differentiation is not common. It may be found in dispersed as well as consolidated steles. It is usually associated with a large pith and a narrow procambium ring. The distribution and significance of this type are fully discussed below, where reasons are given for regarding it as the most advanced condition in dicotyledons. It is illustrated in Figs.24,25,92-94

2). Irregular.

In this type of differentiation the first elements to appear do so as usual on the margin of the procambial strand next the pith. Succeeding elements appear centrifugally, but not necessarily in contact with each other nor with the first-formed elements. They therefor appear scattered and mixed with parenchyma. In these cases there is a fair amount of primary xylem, more so than in the regular type. As soon as the cambium gets into action the xylem takes on a radial seriation. (See Figs. 2, I8-2I).

This irregular type of xylem differentiation is usually associated with leaf traces which fit their gaps less closely, that is with dispersed steles or with consolidated steles of medium degree (I.E. gap-residue parenchyma about 20%.) It is suggested that the procambial strand, being less compressed on all sides, retains its original irregular mosaic and that this is displayed in the pattern of the maturing vascular elements. The periclinal divisions which marke the initiation of cambium develope more or less simultaneously across the whole strand, not(as in the regular type) opposite the xylem first. (See Figs. 36,73,9I.)

The stelar patterns described above may be summed up in the following scheme:-



Symbols used:-

C: consolidated stele (large traces, small gap-residues)
D: dispersed stele (small traces, large gap-residues)
M: multisect stele (leaf trace broken up before differentiation)
R: regular xylem (primary xylem alternates with parenchyma)
I: irregular xylem (primary xylem mixed with parenchyma)
X': xylem precedes cambium in differentiation)
C': cambium precedes xylem in differentiation
O: open secondary stele (gap-residues maintained as rays)
B: bound secondary stele (gap residues closed by joining segments)

The above scheme shows all combinations of the factors considered: some are theoretically impossible, e.g. irregular xylem cannot arise if cambium precedes xylem: these types are deleted /. Other possible types have not been found: these are deleted - .

By using the above symbols it is possible to write a shorthand description of the stelar pattern. E.G. Tilia is CRX'O: Fraxinus is MRX'O: Clematis is DIX'O: Helianthus is DRC'B, etc. These symbols will be used in the conspectus of stelar types now to be given.

Before attempting to assess the evolutionary significance of the types of stele found in the dicotyledons, it will be necessary to give a survey of their distribution throughout the various families. For this purpose a comparison was made of the current systems of classification to see which was the most suitable.

Of the systems currently in use, that of Bentham and Hooker (4) is the oldest. While not professing to be a strictly 'natural' (evolutionary) classification, it nevertheless indicates the broad lines at least among the Polypetalae and the Gamopetalae. The grouping of the apetalous forms is less satisfactory. By beginning the system with the Order Ranales, the authors indicate that they consider hypogynous petaloid types with indefinite free floral parts, spirally arranged, to be the most primitive: it follows that apetalous forms are reduced. The grouping together of all such types at the end of the system is no doubt convenient, but it obscures their relationships to each other and to forms with petals.

Rendle (27) follows the classic Engler and Prantl (12) system in his main lines, notably in beginning with the apetalous forms. He sub-divides many of the large Bentham and Hooker families into more manageable groups. However the inclusion of forms with syncarpous ovaries in the apetalous families shows the artificiality of the grouping.

Hutchinson (16) divides the Bentham and Hooker families still further, creating many unigeneric families. He maintains the position of the Magnoliaceous flower as the primitive type, and he distributes the apetalous families among the polypetalous. This part of his system is satisfactory from the anatomical point of view. As placed by Hutchinson, most of the apetalous families are anatomically consistent with the orders in which they are put. Another interesting feature of this system is the demarcation of

of two more or less distinct lines of descent in the dicotyledons, one line being mainly arboreal and the other mainly herbaceous. Hutchinson regards these lines as of equal antiquity and derives them from the Magnoliales and Ranales respectively. He considers that the more or less woody members of his herbaceous line represent an upward evolution from the herbaceous types. This system shows many advances on those previously suggested, but from the point of view of convenience of presentation his large number of very small families and his 'double-line' conception make it difficult to give a clear picture of the whole.

Accordingly the data to be presented will be arranged according to the system of Bentham and Hooker, with the compromise that the apetalous families will be inserted as nearly as possible according to Hutchinson. From the anatomical point of view this is justified: it will be seen that the stelar types are, in general, consistent.

DISTRIBUTION OF STELAR PATTERNS: CONSPECTUS OF RESULTS.

POLYPETALAE & APETALAE (in brackets).

1). RANALES.
- 1. Ranunculaceae: DIO
  (Ceratophyllaceae: anomalous)
- 2. Dilleniaceae: DIO
- 3. Calycanthaceae: CRX'O
- 4. Magnoliaceae: CIO
- 5. Anonaceae: DIO
  (Monimiaceae: MRO
  Lauraceae: MRO
  Myristicaceae -)
- 6. Menispermaceae: DIO, DIB
  (Cytinaceae: anomal.
  Aristolochiaceae: DIO
  Nepenthaceae -
  Piperaceae: DIB
  Saururaceae -
  Chloranthaceae: CRX'O
  Lacistemaceae -)
- 7. Berberidaceae: DIO
- 8. Nymphaeaceae: DIA

2). PARIETALES.
- 9. Sarraceniaceae -
  (Podostemonaceae: anomal.
  Elatinaceae -)
- 10. Papaveraceae: DIO
- 11. Cruciferae: DIO, DIB
- 12. Capparidaceae: MRO, MRB
- 13. Resedaceae: MRO
- 14. Cistineae: MRO
- 15. Violarieae: DRB
- 16. Canellaceae -
- 17. Bixineae: CRX'O

3). POLYGALINEAE.
- 18. Pittosporeae: CRX'O
- 19. Tremandreae: CRX'O
- 20. Polygaleae: MRO
- 21. Vochysiaceae -

4). CARYOPHYLLINEAE.
- 22. Frankeniaceae: MRO
- 23. Caryophyllaceae: DIA
- 24. Portulacaceae: DIO
  (Polygonaceae: DIO
  Illecebraceae: DRO
  Phytolaccaceae: DIO, DIB
  Chenopodiaceae: DRB anom.
  Batidaceae -
  Amarantaceae: DIO anom.)
- 25. Tamariscineae: DIO

5). GUTTIFERALES.
- 26. Elatinaceae -
- 27. Hypericineae: MRO
- 28. Guttiferae: MRO
- 29. Ternstroemiaceae: MRO
- 30. Dipterocarpae: CRX'O
- 31. Chlaenaceae -

6). MALVALES.
32. Malvaceae:MRO
33. Sterculiaceae:MRB
34. Tiliaceae:CRX'O

7). GERANIALES.
35. Linaceae:CRX'O
36. Humiriaceae -
37. Malpighiaceae:DRO
(Euphorbiaceae:MRO)
38. Zygophyllaceae:CRX'O
39. Geraniaceae:DIB
40. Rutaceae:MRO,CRX'O
4I. Simarubaceae:CRX'O
42. Ochnaceae:MRO
43. Burseraceae:MRO
44. Meliaceae:MRB
45. Chailletiaceae -

8). OLACALES.
46. Olacineae:MRO
47. Ilicineae:CRX'O,DIO
(Empetraceae:CRX'O)
48. Cyrilleae -

9). CELASTRALES.
49. Celastrineae:MRO
(Loranthaceae:DRO
Santalaceae:CRX'O
Balanophoraceae -)
50. Stackhousieae -
5I. Rhmneae:CRX'O,DIO
52. Ampelideae:DIO

I0). SAPINDALES.
53. Sapindaceae:DIO
54. Sabiaceae -
55. Anacardiaceae:CRX'O
(Juglandaceae:MRO)
56. Coriareae:CRX'O
57. Moringeae:DIB

II). ROSALES.
58. Connaraceae -
59. Leguminosae:CRX'O,DRO
60. Rosaceae:CRX'O
6I. Saxifragaceae:CRX'O
62. Crassulaceae:DIO,DIB.
63. Droseraceae -
64. Hamamelideae:CRX'O
(Balanopsideae:anomal.
Salicaceae:CRX'O
Garryaceae:DRX'O
Betulaceae:CRX'O
Fagaceae:CRX'O
Casuarinaceae:DIO
Urticaceae:DRO,BRB
Ulmaceae:CRX'O
Moraceae:DRX'O
Buxaceae:MRO
Platanaceae:DIO
Myricaceae:CRX'O)
65. Bruniaceae -
66. Halorageae: anomal.

I2). MYRTALES.
67. Rhizophoraceae -
68. Combretaceae: CRX'0, MRO
69. Myrtaceae: MRO
70. Melastomaceae: DRB
71. Lythrariaceae: MRO
72. Onagrarieae: CRX'0
(Penaeaceae -
Thymeliaceae: CRX'0
Nyctaginaceae: DIB anom.
Proteaceae: CIO)

I3. PASSIFLORALES.
73. Samydaceae: MRO
74: Loasaceae -
75. Turneraceae: MRO
76. Passifloraceae: CRX'0
77. Cucurbitaceae: DIA
78. Begoniaceae: DIB
79. Datiscaceae -

I4. FICOIDALES.
80. Cacteae: DIO anom.
81. Ficoideae: DRO, DRB anom.

I5. UMBELLALES.
82. Umbelliferae: DIO
83. Araliaceae: DIO, CIO anom.
84. Cornaceae: CRX'0.

GAMOPETALAE.

I). RUBIALES.
85. Caprifoliaceae: DIB, DRC'B
86. Rubiaceae: CRX'0 close-set

2). ASTERALES.
87. Valerianaceae: DRC'B
88. Dipsaceae: DRB
89. Calycereae -
90. Compositae: DRX'B, DRC'B

3). CAMPANALES.
91. Stylideae -
92. Goodenovieae: DRX'0
93. Campanulaceae: DRB

4). ERICALES.
94. Ericaceae: CRX'0 close-set
95. Vacciniaceae: CRX'0
96. Monotropeae -
97. Epacridaceae: CRX'0
98. Diapensiaceae -
99. Lennoaceae -

5). PRIMULALES.
100. Plumbagineae: DIO
101. Primulaceae: DIO
102. Myrsinaceae: DIO

6). EBENALES.
103. Sapotaceae -
104. Ebenaceae: DRB
105. Styraceae: MRO

7). GENTIANALES.
I06. Oleaceae:MRO
I07. Salvadoraceae -
I08. Apocynaceae:MRO
I09. Asclepiadaceae:CRC'B
II0. Loganiaceae: CRX'0,MRO
III. Gentianaceae: DRB,DI0.

8). POLEMONIALES.
II2. Polemoniaceae: DRC'B
II3. Hydrophyllaceae:DIA
II4. Boragineae: DIB
II5. Convolvulaceae:DRC'B
II6. Solanaceae:DRC'B

9). PERSONALES.
II7. Scrophularineae:CRX'0,DIB,DRC'B
II8. Orobanchaceae -
II9. Lentibularieae -
I20. Columelliaceae -
I2I. Gesneraceae:DRB
I22. Bignoniaceae:DRX'B anom.
I23. Pedalineae -
I24. Acanthaceae:DRX'B

I0). LAMIALES.
I25. Myoporineae:MRO
I26. Selagineae:DRB
I27. Vervenaceae:DRC'B
I28. Labiatae:DRC'B

I29. Plantagineae:DRO

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SYSTEMATIC SURVEY:

CATALOGUE OF FAMILIES & GENERA EXAMINED.

Arranged according to the system of Bentham and Hooker (4) with the 'Apetalae' (in brackets) inserted according to Hutchinson(16).

Order I. RANALES.

I. Ranunculaceae.
Herbaceous to softly woody. Phyllotaxis spiral to decussate. Leaves usually large, often compound with large sheathing pulvini. Leaf-gap trilacunar.

Genera examined: Delphinium, Thalictrum, Ranunculus, Nigella, Cimicifuga, Clematis.
Stelar type: DIO.

In all the Ranunculaceae there is a high proportion of primary xylem in the vascular bundles, scattered among the parenchyma. The development of interfascicular cambium varies: in the slender annual stems there is none while a Clematis has a complete ring, functional for many years. The sscondary stele is open, the interfascicular parenchyma segments being maintained. In Clematis they are lignified and organised as rays. In some marsh types (e.g. Ranunculus Flammula) the vascular cambium ceases to function at an early stage.

(Ceratophyllaceae. Anomalous aquatics with reduced central steles).

2. Dilleniaceae.
Woody: leaves alternate:leaf-gap trilacunar.

Genera examined: Dillenia, Hibbertia.
Stelar type: DIO.

The one-year old stem shows a ring of rather narrow bundles with a fair proportion of irregular primary xylem: gap-residues are wide and maintained as rays in older stems.

3. Calycanthaceae.
Woody:leaves opposite: leaf-gap trilacunar.

Genus examined: Calycanthus.
Stelar type: CRX'O.

4. Magnoliaceae.
Entirely arboreal or shrubby (some woody climbers): phyllotaxis spiral: leaf-gap uni-,tri-, or multilacunar.

Generae examined: Drymis, Illycium, Liriodendron, Magnolia, Schizandra.
Stelar type: CIO.

The vascular strands contain a considerable amount of irregular primary xylem and in the young stem strongly resemble the Ranunculaceous bundle. The combination of a consolidated stele with irregular primary xylem is unique in the Dicotyledons. The peculiar histology of mature xylem is considered another point in favour of the antiquity of this Family.

5. Anonaceae.
Woody: leaves two-ranked:leaf-gap unilacunar.

Genera examined: Polyalthia, Monodora.
Stelar type: DIO.

The chief interest of the stele here is the splitting of the original bundles by wedges of parenchyma which appear even in the first year's growth. The bundles are divided and re-divided in successive season's growth, resulting in a highly parenchymatous stele.

(Monimiaceae.
Woody:leaves opposite:leaf-gap unilacunar.
Genus examined:Peumus.
Stelar type: MRO.

Lauraceae.
Woody:leaves alternate:leaf-gap unilacunar.
Genus examined: Persea.
Stelar type: MRO.

Myristicaceae: not obtainable.)

6. Menispermaceae.
Mostly twining shrubs, some herbs,some trees: leaves alternate: leaf-gap trilacunar.

Genera examined: Anamirta, Cocculus,Cyclea,Stephania.
Stelar types: DIO,DIB.

The liane types (Cocculus, Cyclea) show typical structure with very large vessels and well-marked pericyclic sclerenchyma.

(Aristolochiaceae.
Mostly lianes: leaves alternate:leaf-gap trilacunar.
Genus examined: Aristolochia.
Stelar type: DIO.

Piperaceae.
Herbaceous to shrubby: leaves alternate: leaf-gap tri- or multilacunar.
Genera examined: Piper, Peperomia.
Stelar types: DIB,DIO.

These genera have anomalous stem structure but the bundles which take part in secondary thickening are normal.

Chloranthaceae.
Herbaceous to woody:leaves opposite:leaf-gap tri- or multilacunar.
Genus examined: Chloranthus.
Stelar type: CRX'O.

Cytinaceae,Nepenthaceae, Saururaceae:
aberrant morphology; not examined.)

7. Berberidaceae.
Herbaceous perennials-shrubs: leaves alternate: leaf-gap tri- or multilacunar.
Genera examined: Berberis, Epimedium,Sargentodoxa.
Stelar type: DIO.

Even in Berberis, the most woody, the bundles are widely spaced, and reinforced by lignification of pith border and later the rays: also pericyclic sclerenchyma.

8. Nymphaeaceae.
Aquatics:leaves alternate.

Genera examined: Nymphaea, Castalia.
Stelar type: DIO.

Considerable modification of the stem anatomy associated with submerged habit. Peduncle shows great development of air spaces and reduced amount of xylem.

Order 2. PARIETALES.

9. Sarraceniaceae.
Herbaceous with radical leaves,insectivorous.Not examined.

(Podostemonaceae: anomalous, not examined.)

IO. Papaveraceae (inc. Fumariaceae).
Herbaceous -shrubby:leaves alternate:leaf-gap trilacunar.

Genera examined: Papaver, Chelidonium, Eschscholtzia, Meconopsis, Romneya, Fumaria, Corydalis.
Stelar type:DIO.

The herbaceous and annual types show no secondary thickening: instead the stems are reinforced by lignified parenchyma in pericycle and between bundles. The shrub types are similar but a cambium ring is present. Eschscholtzia and Fumaria have markedly 'Ranunculaceous' bundles.

II. Cruciferae.
Herbs, a few undershrubs,many perennial: leaves alternate; leaf-gap tri- and unilacunar.

Genera examined: Matthiola, Cheiranthus, Alyssum Capsella, Crambe, Nasturtium.
Stelar types: DIO, DIB.

Forms with secondary thickening show a continuous ring of lignified tissue, rather close and uniform. Annuals show lignification of pith border linking inner points of xylem together.

I2. Capparidaceae.
Herbs and shrubs:leaves alternate:leaf-gap unilacunar.

Genera examined: Capparis, Steriphoma, Tovaria.
Stelar types: MRO, MRB.

Capparis is an example of extreme multisection of the stele: the original vascular segments are very narrow, often only a single file of xylem. Secondary thickening binds these narrow strands together with a close,uniform lignified tissue,mainly tracheidal.

I3. Resedaceae.
Herbaceous:leaves alternate: leaf-gap unilacunar.

Genus examined: Reseda.
Stelar type: MRO.

I4. Cistineae.
Herbaceous-woody: leaves opposite:leaf-gap unilacunar.
Genera examined: Cistus, Helianthemum.
Stelar type: MRO.

I5. Violarieae.
Herbaceous-woody: leaves alternate:leaf-gap trilacunar.
Genus examined:Viola.
Stelar type: DRB.

I6. Canellaceae.
Woody:leaves alternate.
Not obtainable.

I7. Bixineae.
Woody:leaves alternate:leaf-gap trilacunar.
Genus examined:Bixa.
Stelar type: CRX'O.
Closely resembles Tilia.

Order 3. POLYGALINEAE.

I8. Pittosporeae.
Trees or shrubs:leaves alternate:leaf-gap trilacunar.
Genera examined:Pittosporum, Solleya.
Stelar type: CRX'O.

I9. Tremandreae.
Herbaceous:leaves opposite or whorled:leaf-gap unilacunar.
Genera examined: Platytheca, Tetratheca.
Stelar type: CRX'O.

20. Polygaleae.
Herbaceous-woody:leaves various:leaf-gap unilacunar.
Genus examined:Polygala.
Stelar type: MRO.

2I. Vochysiaceae.
Not obtainable.

Order 4. CARYOPHYLLINEAE.

22. Frankeniaceae.
Herbaceous(halophytic):leaves opposite.
Genus examined:Frankenia.
Stelar type: MRO.

23. Caryophylleae.
Herbaceous:leaves opposite:leaf-gap unilacunar.
Genera examined: Lychnis, Stellaria.
Stelar type: DIO.
The annual forms show no secondary thickening:compensating lignification of the pericycle and interfascicular parenchyma very well marked.

24. Portulaceae.
Annual herbs,often fleshy:opposite leaves:leaf-gap single.
Genera examined:Claytonia,Calandrinia.
Stelar type: DIO.

25. Tamariscineae.
Shrubs or trees:leaves alternate:
Genus examined:Tamarix.
Stelar type: DIO.

The stem of Tamarix is interesting. Although a perennial of shrubby growth, the stem is soft to cut on account of the large amount of parenchyma in the stele. The original bundles are widely separated and secondary growth maintains wide parenchymatous rays as well as much xylem parenchyma.

(Polygonaceae.
HerbaceousLleaves alternate:leaf-gap multilacunar.
Genus examined:Polygonum.
Stelar type DIO.

The anatomy of these stems is complex. Many bundles enter the stele at each node and in addition there are several anomalous features, e.g. medullary bundles, additional bundles developed in the pericycle etc. The structure and arrangement of the true foliar bundles corresponds however to the DIO pattern.

Illecbraceae.
Herbaceous: leaves opposite:leaf-gap unilacunar.
Genus examinedL Illecebrum.
Stelar type: DRO.

Phytolaccaceae.
Herbaceous to woody:leaves alternate:leaf-gap unilacunar.
Genera examined; Petiveria,Rivina,Villamilla.
Stelar type:DIO.
Anomalous ring of bundles in the pericycle.

Chenopodiaceae.
Herbaceous(halophytic) a few woody:leaves alternate:leaf-gap unilacunar.
Genera exmmined: Chenopodium Salsola.
Stelar type: DRB(anomalous).

Batidaceae.
Woody:leaves opposite.
Only genus Batis, not obtainable.

Amarantaceae.
Herbaceous-shrubby: leaves opposite or alternate:leaf-gap unilacunar.
Genus examined; Amaranthus.
Stelar type:DIO.(Anomalous).

Order 5. GUTTIFERALES.

26. Elatineae.
Undershrubs,herbs or annuals(water):leaves opposite or whorled:

27. Hypericineae.
Herbaceous:leaves opposite:leaf-gap unilacunar.
Genus examined:Hypericum.
Stelar type:MRO.

28. Guttiferae.
Woody:leaves opposite:leaf-gap unilacunar.
Genus examined: Clusia.
Stelar type MRO.

29. Ternstroemiaceae.
Woody:leaves alternate:leaf-gap unilacunar.
Genera examined: Camellia,Thea,Stuartia.
Stelar types:MRO,CRX'O.
This family shows clearly the connexion between these two types of stele. Stuartia has a more compact stem, with smaller pith and moderately deep stelar ring;its type is CRX'O. Camellia and Thea have larger stems, bigger piths, narrower stelar rings; they are MRO. This bears out the suggestion that MRO is an expansion of CRO.

30. Dipterocarpae.
Trees:leaves alternate: leaf-gap tri- or multilacunar.
Genus examined: Shorea.
Stelar type: CRX'O.

31. Chlaenaceae. not obtainable.

Order 6. MALVALES.

32. Malvaceae.
Herbs, shrubs and trees:leaves alternate:leaf-gap trilacunar.
Genera examined: Hibiscus, Malva, Lavatera.
Stelar type: MRO.

33. Sterculiaceae.
Herbs, shrubs and trees:leaves alternate:leaf-gap trilacunar.
Genusexamined: Hermannia.
Stelar type: MRB.

34. Tiliaceae.
Trees and shrubs:leaves distichous:leaf-gap trilacunar.
Genera examined: Tilia,Sparmannia, Aristotelia, Vallaea, Tricuspidaria,Elaeocarpus, Luehia, Grewia,Corchorus.
Stelar type: CRX'O.

Order 7. GERANIALES.

35. Linaceae.(incl. Erythroxylaceae).
Herbaceous to shrubby:leaves alternate:leaf-gap trilacunar.
Genera examined:Linum, Reintwardia,Erythroxylum.
Stelar type: CRX'O.
The mature wood in these types is very fegular with narrow secondary rays. Bast fibres well developed in Linum, less in others.

36. Humiriaceae. not obtainable.

37. Malpighiaceae.
Woody,usually climbing:leaves opposite:leaf-gap trilacunar.
Genera examined: Malpighia, Banisteria.
Stelar type: DRO.
Some anomalous forms among the lianes.

38. Zygophyllaceae.
Woody:opposite leaves: leaf-gap trilacunar.
Genera examined:Bulnesia, Guaiacum.
Stelar type: CRX'O.

39. Geraniaceae.
Herbaceous:leaves alternate:leaf-gap trilacunar.
Genera examined: Geranium,Pelargonium.
Stelar type: DIB.
Oxalis bupleurifolium has stele CRX'0, Oxalis Ortegii DRA.

(Euphorbiaceae.
Vegetative habit very varied.
Genera examined: Euphorbia, Hevea.
Stelar types: DRO,MRO respectively.)

40. Rutaceae.
Woody:leaves alternate or opposite:leaf-gap trilacunar.
Genera examined: Citrus, Ruta,Murraya,Skimmia.
Stelar types: MRO,CRX'O.

41. Simarubaceae.
Woody:alternate leaves:leaf-gap multilacunar (7).
Genera examined: Harrisonia, Picramnea.
Stelar types: MRB,CRX'O.

42. Ochnaceae.
Woody:alternate leaves: leaf-gap trilacunar.
Genus examined:Ochna.
Stelar type: MRO.

43. Burseraceae.
Woody;alternate leaves: leaf-gap multilacunar (5)
Genus examined: Bursera.
Stelar type:MRO.

44. Meliaceae.
Woody:alternate leaves:leaf-gap multilacunar (5).
Genus examined:Trichelia.
Stelar type: MRB.

45. Chailletiaceae. not obtainable.

Order 8. OLACALES.

46. Olacineae.
Woody:alternate leaves: leaf-gap unilacunar.
Genera examined:Apodytes, Villaresia.
Stelar types: MRO,DIO.

47. Ilicineae.
Woody:alternate leaves:leaf-gap unilacunar.
Genus examined: Ilex.
Stelar type:CRX'O.

48. Cyrilleae. not examined.

Order 9. CELASTRALES.

49. Celastrineae.
Woody:leaves opposite:leaf-gap unilacunar.
Genera examined: Euonymus, Cassine, Catha.
Stelar type: MRO.

(Loranthaceae.
Small semi-parasitic shrubs with green leaves:leaves opposite: leaf-gap trilacunar.
Genus examined:Viscum.
Stelar type:DRX'O.
Stem structure very like that of Santalum, but vascular segments more widely spaced.

Santalaceae.
Semi-parasitic woody plants:leaves opposite:leaf-gap unilacunar.
Genus examined:Santalum
Stelar type: CRX'O.

Balanophoraceae.
Root-parasites lacking chlorophyll, with reduced leaves.
Not examined, but according to Solereder (39) the bundles in the peduncle are isolated collateral; they pursue an irregular course in the rhizome. The stem structure is ovbiously highly anomalous.)

50. Stackhousieae. not obtainable.

51. Rhamnae.
Woody:leaves alternate:leaf-gap trilacunar.
Genera examined: Ceanothus, Phylica, Colletia.
Stelar types: CRX'O, DIO(Colletia.)
The anatomy of Colletia ferox (the species examined) is an interesting contrast to the other two genera. They are normal shrubs in habit, while Colletia is peculiar. It has in each axil two serial buds; the upper gives a triangular 'thorn', the lower, flowers or a branch of unlimited growth. The apparently xerophytic appearance is not upheld by the anatomy, except for a very thick cuticle. The stele is dispersed, and in the first year's growth the pericyclic fibres give a cellulosic reaction. (Cf. Spartium, Leguminosae, below).

(Elaeagnaceae.
Woody:leaves opposite or alternate, leathery:leaf-gap unilacunar.
Genus examined: Hippophae.
Stelar type: MRO.)

52. Ampelideae.
Woody climbers: leaves alternate: leaf-gap tri- or multilacunar.
Genera examined: Vitis, Ampelopsis.
Stelar type: DIO.

Order IO. SAPINDALES.

53. Sapindaceae.
Trees and woody lianes: leaves alternate:leaf-gap trilacunar.
Genera examined: Aesculus, Serjania.
Stelar type: DIO.
Serjania shows anomalous structure.

54. Sabiaceae. not examined.

55. Anacardiaceae.
Woody:alternateleaves: leaf-gap trilacunar.
Genus examined: Rhus.
Stelar type: CRX'O.

(Juglandaceae.
Woody:alternate leaves: leaf-gap trilacunar.
Genus examined: Juglans.
Stelar type:MRO.

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56. Coriareae.
Woody:leaves opposite:leaf-gap unilacunar.
Genus examined: Coriaria
Stelar type CRX'O.

57. Moringae.
Woody:leaves alternate.
Genus examined: Moringa.
Stelar type: DIB.

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Order II. ROSALES.

58. Connaraceae. Not examined.

59. Leguminosae.
Habit various: leaves alternate: leaf-gap trilacunar.
Genera examined: Coronilla, Cytisus, Lupinus, Spartium, Trifolium, Vicia, Wistaria.
Stelar types: DIO,DIB, DRO,DRB,CRX'O.
It will be seen that there is a considerable range of stelar type in this Family. In general the steles accord well with the habit-types of the forms studied. Wistaria, which was the most woody example, had the regular close-set consolidated stele. Vicia Faba with its soft stem is DIO. The harder creeping stem of Trifolium is DRB. Spartium junceum, with its smooth green rush-like stem, is of the type DRO, with joining lignified parenchyma and very beautiful cellulosic pericyclic fibres, in addition to palisade assimilating cortical parenchyma.(Cf. Colletia ferox, above.)

60. Rosaceae.
Tress, shrubs and herbs,mostly perennial:leaves alternate: leaf-gap trilacunar.
Genera examined: Alchemilla, Cotoneaster, Crataegus, Dryas, Geum, Potentilla, Prunus, Pyrus, Rosa, Rubus, Spiraea.
Stelar type: CRX'O.
The stelar structure in this Family is very uniform.

(Calycanthaceae.
Woody:opposite leaves: leaf-gap trilacunar.Stele CRX'0.
As noted above under Ranales, Hutchinson inserts this Family here: it fits the anatomical scheme better than under Ranales.)

61. Saxifragaceae.
Mostly herbaceous, a few trees, many xerophytes: leaves usually alternate:leaf-gap tri- and multilacunar.
Genera examined: Saxifraga, Chrysosplenium, Philadelphus, Hydrangia, Deutzia, Tolmeia, Escallonia, Ribes.
Stelar type: CRX'0 (except Chrysosplenium.)
Chrysosplenium has a peculiar reduced central stele.

62. Droseraceae.

64. Hamamelideae.
Woody:leaves alternate:leaf-gap trilacunar.
Genus examined: Hamamelis.
Stelar type: CRX'0.

(Buxaceae.
Woody:alternate leaves:leaf-gap unilacunar.
Genus examined:Buxus.
Stelar type: MR0.

Platanaceae.
Woody:leaves two-ranked:leaf-gap multilacunar (7).
Genus examined:Platanus.
Stelar type: DI0.

Salicaceae.
Woody:leaves alternate:leaf-gap trilacunar.
Genera examined:Salix,Populus.
Stelar type: CRX'0.

Garryaceae.
Woody shrubs:leaves opposite:leaf-gap trilacunar.
Genus examinee: Garrya.
Stelar type:DR0.

Myricaceae.
Woody:leaves alternate:leaf-gap trilacunar.
Genus examined: Myrica.
Stelar type: CRX'0.

Betulaceae.
Woody:leaves alternate: leaf-gap trilacunar.
Genera examined: Betula, Alnus.
Stelar type: CRX'0.

Corylaceae.
Woody: leaves alternate: leaf-gap trilacunar.
Genera examined: Corylus, Carpinus.
Stelar type: CRX'0.

Fagaceae.
Woody: leaves alternate: leaf-gap trilacunar.
Genera examined: Fagus, Quercus. CRX'0.

Casuarinaceae.
Trees of strongly xerophytic habit:leaves reduced,whorled: leaf-gap unilacunar.
Genus examined: Casuarina.
Stelar type: DIO.
Casuarina shows interesting anatomical structufe -grooved stem,thick cuticle,palisade-like cortex- but the stele is normal.

Ulmaceae.
Woody:leaves alternate:leaf-gap trilacunar.
Genus examined: Ulmus.
Stelar type:CRX'O.

Moraceae.
Woody:leaves alternate; leaf-gap tri- or multilacunar (5).
Genera examined: Ficus,Dorstenia.
Stelar type:DRX'O.

Urticaceae.
Mostly herbaceous:leaves alternate or opposite:leaf-gap trilacunar.
Genera examined: Urtica, Boehmeria.
Stelar types: DIB,DRX'B.

Cannabinaceae.
Herbaceous:leaves alternate or opposite:leaf-gap trilacunar.
Genus examined:Humulus.)

65. Bruniaceae. Not obtainable.

66. Haloragèae.
Aquatic herbs:leaves various.
Genera examined: Myriophyllum, Gunnera.
Stelar types: anomalous. Myriophyllum has reduced centralised stele, Gunnera shows 'polystely.'

The general anatomy in the Order Rosales is consistent, the dominant type being the regular consolidated stele. Divergences occur mainly in the herbaceous types which show a greater development of parenchyma. The anomalous reduced steles in Haloragèae are obviously correlated with the aquatic habit. The close resemblance between the steles of Salicaceae, Fagaceae,Myricaceae and the woody Rosaceae support their inclusion at this point of the system rather than at the end(Bentham and Hooker) or the beginning (Engler, Rendle).

Order I2. MYRTALES.

67. Rhizophoraceae. Not obtainable.

68. Combretaceae.
Woody:leaves mainly opposite:leaf-gap unilacunar.
Genera examined: Combretum, Quisqualis.
Stelar types: MRS,CRX'O.

69. Myrtaceae.
Woody:leaves opposite:leaf-gap unilacunar.
Genera examined: Backhousia, Eugenia, Leptospermum, Psidium.
Stelar type: MRO.
This Family shows very uniform stelar structure.

70. Melastomaceae.
Herbaceous to woody:leaves opposite:leaf-gap unilacunar.
Genus examined:Tibouchina.
Stelar type: DRB(anomalous).

71. Lythrarieae.
Herbaceous to woody:leaves opposite or whorled:leaf-gap unilacunar.
Genus examined:Lythrum.
Stelar type:MRO.

72. Onagrarieae.
Herbaceous:leaves opposite or alternate:leaf-gap unilacunar.
Genera examined:Clarkia,Epilobium,Fuchsia,Godetia.
Stelar type: CRX'O close-set.

(Thymeliaceae.
Woody:leaves opposite or alternate:leaf-gap unilacunar.
Genus examined:Daphne.
Stelar type: CRX'O.
The systematic position of this family is difficult.

Nyctaginaceae.
Herbaceous to woody:leaves alternate or opposite:leaf-gap unilacunar.
Genus examined: Bougainvillea.
Stelar type: DIB(anomalous).

Proteaceae.
Woody:leaves alternate:leaf-gap trilacunar.
Genera examined: Grevillea, Hakea.
Stelar type:CIO.)

Order I3. PASSIFLORALES.

73. Samydaceae.
Woody:leaves alternate.
Genus examined: Samyda.
Stelar type:MRO.

74. Loasaceae.
Herbaceous:not obtainable.

75. Turneraceae.
Herbaceous to woody:leaves alternate.
Genus examined:Turnera.
Stelar type: MRO.

76. Passifloraceae.
Shrubs and herbs, mostly climbing:leaves alternate:leaf-gap trilacunar.
Genus examined:Passiflora.
Stelar type:CRX'O.

77. Cucurbitaceae.
Climbing annual herbs:leaves alternate:leaf-gap trilacunar.
Genera examined: Cucurbita,Cyclanthera,Gurania.
Stelar type:DIO.

78. Begoniaceae.
Herbaceous:leaves alternate:leaf-gap multilacunar (5).
Genus examined:Begonia. Stelar type:DIB.

79. Datiscaceae:not obtainable.

Order I4. FICOIDALES.

80. Cactaceae.
Succulent herbs and shrubs of varied habit:leaves usually very much reduced. The extreme succulence of many Cacti makes the stem structure highly distorted and peculiar, but the individual bundles correspond to the dispersed irregular type.

8I. Ficoideae (Aizoaceae).
Herbs or low shrubs, often fleshy:leaves alternate or opposite: leaf-gap unilacunar.
Genus examined:Mesembryanthemum.
Stelar type:anomalous,original bundles apparently DR.

Order I5. UMBELLALES.

82. Umbelliferae.
Herbaceous:leaves alternate:leaf-gap multilacunar.
Genera examined: Astrantia,Foeniculum,Oenanthe.
The Umbelliferae show much variation in details of structure, especially in the mechanical arrangements for securing stability, e.g. lignified joining parenchyma, discrete or continuous pericyclic fibres, collenchyma etc. Many show anomalous cortical or medullary bundles. A successful adaptation of the highly dispersed stele, correlated with the large leaf-size and multiplex leaf-trace.

83. Araliaceae.
Mostly woody,sometimes climbing:leaves alternate:leaf-gap multilacunar.
Genera examined: Aralia, Hedera, Panax.
Stelar types: DIO,CIO, anomalous.

84. Cornaceae.
Woody:leaves opposite or alternate:leaf-gap trilacunar.
Genera examined: Aucuba, Cornus, Griselinia.

The anatomy of Cornaceae is simpler and more straightforward than that of Umbelliferae and Araliaceae. From this point of view it is the least advanced Family of the Order,.

GAMOPETALAE.

Order I. RUBIALES.

85. Caprifoliaceae.
Mostly shrubby:leaves opposite:leaf-gap trilacunar (except Sambucus, 5 gaps).
Genera examined: Diervilla, Leycesteria, Linnaea, Sambucus, Symphoricarpus, Viburnum.
Stelar types: DRO, DIO (Sambucus).

86. Rubiaceae.
Mostly woody, some herbs:leaves opposite or verticillate: leaf-gap unilacunar.
Genera examined: Crucianella, Galium, Ixora, Rondeletia.
Stelar type: CRX'O very close-set.

Order 2. ASTERALES.

87. Valerianaceae.
Herbaceous:leaves opposite or radical:leaf-gap trilacunar.
Genus examined: Centranthus
Stelar type: DRC'B.

88. Dipsaceae.
Herbaceous:leaves opposite or verticillate:leaf-gap trilacunar.
Genus examined:Scabiosa.
Stelar type:DRB.

89. Calycerae:not obtainable.

90. Compositae.
Herbaceous:woody:leaves alternate or opposite:leaf-gap trilacunar(sometimes multilacunar.)
Genera examined: Centaurea, Chrysanthemum, Gazania, Helianthus, Senecio, Olearia.
Stelar type: DRC'B, DRX'B(in more woody forms).

Order 3. CAMPANALES.

91. Stylideae:not obtainable.

92. Goodenovieae.
Herbs or undershrubs:leaves alternate:leaf-gap tri- or multilacunar.
Genera examined: Goodenia, Selliera.
Stelar type: DRX'O anomalous.

93. Campanulaceae.
Mostly herbaceous:leaves alternate:leaf-gap unilacunar.
Genera examined: Campanula, Lobelia, Phyteuma.
Stelar type: DRB.

Order 4. ERICALES.

94. Ericaceae.
Woody:leaves alternate:leaf-gap unilacunar.
Genera examined: Erica, Gaultheria, Ledum, Rhododendron.
Stelar type: CRX'O very close-set.

95. Vacciniaceae.
Woody:leaves alternate:leaf-gap unilacunar.
Genus examined:Vaccinium.
Stelar type: CRX'O.

96. Monotropae.
Leafless parasitic herbs without chlorophyll. Not examined, highly anomalous.

97. Epacridaceae.
Woody:leaves alternate:leaf-gap multilacunar.
Genus examined: Cyathodes.
Stelar type CRX'O.

98. Diapensiaceae:not obtainable.

99. Lennoaceae:not obtainable.

Order 5. PRIMULALES.

I00. Plumbagineae.
Herbs,undershrubs or climbers:leaves alternate or radical: leaf-gap trilacunar.
Genera examined: Armeria, Limonium, Plumbago.
Stelar type:DIO,

I0I. Primulaceae.
Herbaceous:leaves mostly basal:leaf-gap unilacunar.
Genera examined:Anagallis,Glaux,Primula.
Stelar type:DIO.
The peduncle of Primula Auricula shows 'polystely'.

I02. Myrsinaceae.
Woody:leaves alternate:leaf-gap unilacunar.
Genera examined:Ardisia,Deherainea,Myrsine.
Stelar type:DIO.
In spite of their woody habit, the stems of these types show much parenchyma in the steles: they are hard to cut because of a strongly lignified pericycle.

Order 6. EBENALES.

I03. Sapotaceae: not examined.

I04. Ebenaceae.
Woody:leaves alternate:leaf-gap unilacunar.
Genus examined: Diospyros.
Stelar type: DRX'B.

I05. Styraceae.
Woody:leaves alternate:leaf-gap unilacunar.
Genus examined:Styrax.
Stelar type MRO.

Order 7. GENTIANALES.

I06. Oleaceae.
Woody:leaves opposite:leaf-gap unilacunar.
Genera examined: Fraxinus,Jasminum, Ligustrum, Olea, Syringa.
Stelar type:MRO.

I07. Salvadoraceae:not obtainable.

108. Apocynaceae.
Trees, shrubs or climbers, rarely perennial herbs: leaves opposite: leaf-gap unilacunar.
Genera examined: Edhites, Nerium, Vinca.
Stelar type: MRO.

109. Asclepiadaceae.
Perennial herbs, undershrubs or shrubs: leaves opposite: leaf-gap unilacunar.
Genera examined: Asclepias, Ceropegia, Pergularia.
Stelar type: CRC'B.

110. Loganiaceae.
Woody: leaves opposite: leaf-gap unilacunar.
Genera examined: Buddleia, Desfontainea, Strychnos.
Stelar types: MRO, CRX'O.
The mature stem of Strychnos shows the anomaly of 'phloem islands' enclosed in the secondary xylem.

111. Gentianaceae.
Herbaceous: leaves opposite: leaf-gap unilacunar.
Genera examined: Chironia, Gentiana, Menyanthes.
Stelar types: DRO, DIO.
Menyanthes has an interesting stem, with aerenchyma in the cortex, an endodermis, and widely spaced bundles with irregular xylem.

Order 8. POLEMONIALES.

112. Polemoniaceae.
Mostly herbaceous: leaves alternate or opposite: leaf-gap unilacunar.
Genera examined: Coboea, Phlox, Polemonium.
Stelar type: DRB.
Coboea, being a quick-growing climber, shows precocious cambium (stele DRC'B) and huge vessels.

113. Hydrophyllaceae.
Herbaceous: leaves radical or alternate: leaf-gap unilacunar.
Genera examined: Nemophila, Phacelia.
Stelar type: DIA.
Both show well-marked endodermis.

114. Boragineae.
Herbs, shrubs or trees: leaves alternate: leaf-gap unilacunar.
Genera examined: Anchusa, Myosotis, Symphytum.
Stelar type: DIB.

115. Convolvulaceae.
Herbaceous or woody, often climbing: leaves alternate: leaf-gap unilacunar.
Genera examined: Convolvulus, Ipomoea.
Stelar type: DRC'B.

116. Solanaceae.
Herbaceous of woody: leaves alternate: leaf-gap unilacunar.
Genera examined: Atropa, Petunia, Physalis, Salpiglossis Schizanthus, Solanum.
Stelar type: DRB.
In the more rapidly growing types there is precocious cambium, giving stele DRC'B (confirmed by study of differentiation). Even in the more woody, slower growing types the amount of primary xylem is very small: possibly the whole Family should be grouped DRC'B.

Order 9.PERSONALES.

II7. Scrophularineae.
Herbs or shrubs:leaves alternate or opposite:leaf-gap unilacunar.
Genera examined: Calceolaria, Digitalis, Euphrasia, Linaria, Mimulus, Nemesia, Pentstemon, Scrophularia, Veronica.
Stelar types: CRB, DRB.

The degree of dispersion of the vascular segments is clearly correlated in this Family with the growth-form of the type. E.G. Mimulus is the most dispersed: the relatively massive, large-pithed stems of Digitalis and Scrophularia are more dispersed than the compact stems of Veronica or Euphrasia(CRC'B and CRX'B respectively). Scrophularia and Veronica show precocious cambium.

II8. Orobanchaceae.
Parasitic non-green herbs:leaves alternate, scaly:leaf-gap unilacunar.

II9. Lentibulariaceae.
Herbaceous:leaves radical:leaf-gap unilacunar.

The anatomy of this Family is much modified. According to Solereder(39) some species of Pinguicula show polystelic structure: the peduncle of Utricularia shows a minute axial strand or a vascular ring of alternating xylem and phloem strands.

I20. Columelliaceae: not obtainable.

I2I. Gesneraceae.
Herbs or shrubs:leaves radical or opposite:leaf-gap unilacunar (sometimes tri- or multilacunar).
Genera examined: Aeschynanthus, Columnea, Isoloma, Streptocarpus.
Stelar type: DRB.

I22. Bignoniaceae.
Woody, sometimes climbing:leaves opposite:leaf-gap unilacunar.
Genera examined: Bignonia, Tecoma.
Stelar type: DRX'B anomalous.

I23. Pedalineae: not examined.

I24. Acanthaceae.
Herbaceous or climbing, rarely shrubby:leaves opposite: leaf-gap unilacunar.
Genera examined: Acanthus, Jacobinia, Ruellia.
Stelar type: DRX'B.

Order IO. LAMIALES.

I25. Myoporineae.
Woody:leaves alternate: leaf-gap unilacunar.
Genus examined: Myoporum.
Stelar type: MRO.

I26. Selagineae.
Herbs or undershrubs:leaves alternate.
Genera examined: Selago,Hebenstretia.
Stelar type: DRB.

I27. Verbenaceae.
Herbaceous or woody: leaves opposite or whorled:leaf-gap unilacunar.
Genus examined: Verbena.
Stelar type: DRC'B.

I28. Labiatae.
Herbaceous or rarely woody:leaves opposite or whorled: leaf-gap unilacunar.
Genera examined: Coleus,Lamium,Nepeta,Phlomis, Lavandula,Rosmarinus.
Stelar type: DRB.
Again (as in Scrophularineae) the quick-growing herbaceous types show a precocious cambium,(stele DRC'B) while the more woody are DRX'B.

I29. Plantagineae.
Herbaceous:leaves radical,alternate or opposite: leaf-gap trilacunar.
Genera examined: Littorella,Plantago.
Stelar type: DRO, but very much distorted owing to the rosette habit.

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DISTRIBUTION OF STELAR PATTERNS: DISCUSSION.

The anatomical characters of a plant cannot be considered alone: they must be correlated with its general morphology and floral organisation. The classification of the flowering plants is primarily based on floral characters, and, while systematists differ about details, the main lines of advance are generally accepted. Accordingly the data already given on the distribution of stelar patterns in the Dicotyledons will now be analysed to see if correlations exist between anatomical structure, morphology and systematic position.

It was not possible to examine reppesentatives of every family listed by Bentham and Hooker: some are not in cultivation in this country and under war conditions could not be obtained from abroad. (Herbarium material is of little use where differentiation is to be studied.) No attempt was made to secure some of the highly abnormal parasitic families: their general morphology is so peculiar that their anatomical structure must be considered as quite outside the normal of the group. Taking these things into account, however, it was felt that a sufficient bulk of material had been studied to warrant some general conclusions, namely:-

I). There is a great increase in the number of families wholly or mainly herbaceous in the Gamopetalae:

Woody:herbaceous is 2:1 in Polypetalae Apetalae,
" " " I:I " Gamopetalae.

2). There is an increase in cyclic symmetry (opposite or whorled leaves) in the Gamopetalae:

Spiral : cyclic is 3.4:I in Polypetalae Apetalae,
" " " 2.4:I " Gamopetalae.

3). Dispersion of the stele increases in the Gamopetalae:

Consolidated:dispersed is I.5:I in Polypetalae Apetalae,
" " " I.0:2.4 " Gamopetalae.

4). Regular differentiation of xylem increases in the Gamopetalae:

Irregular : regular is I:I.7 in Polypetalae Apetalae,
" " " I:3 " Gamopetalae.

5). The bound type of stele increases in the Gamopetalae:

Open stele:bound stele is 4:1 in Polypetalae Apetalae,
" " " I:I " Gamopetalae.

It would appear that the arboreal habit, spiral phyllotaxis and consolidated open steles are characteristic of the section showing less advanced floral organisation, while herbaceous habit, cyclic phyllotaxis and dispersed regular bound steles are more common in the section showing the most advanced floral organisation.

The differences in habit will be considered first. The question of the relative antiquity of the arboreal and herbaceous habit in the dicotyledons is of course a major one. Arguments in the past have mainly been drawn from comparative morphology, involving both existing groups and the fossil record. It is generally accepted that Lepidodendron and other fossil Lycopods which show secondary thickening represent ancestral types of which Lycopodium is a relic. The centripetal primary and centrifugal secondary xylem of Lepidodendron are now represented in Lycopodium only by the former. The Calamites-Equisetum relation could be quoted, and also the fact that there are no herbaceous gymnosperms. All these types represent a lower horizon of reproductive efficiency than the angiosperms: that is admitted, but these arguments drawn from morphology in favour of a primitive massive habit are only arguments by analogy. Direct comparison of the anatomy of these types (except the gymnosperms) with the angiosperms are not valid, because the Lycopodian stele has no leaf-gaps and the Equisetum stele is cauline. Moreover such evidence on the differentiation of these types as is available (44) indicates a radically different method from that of the dicotyledon.

Another argument which is often used is the general one, that a large unspecialised structure is more primitive than a small specialised one, the underlying idea really being that it is easier to imagine reduction and contraction than expansion. This is acceptable in the case of the flower, but not in the case of tree-versus-

herb, because a similar precision of phyllotaxis and branching characterizes both: the difference is fundamentally one of scale not of architecture.

Genetics offers another and more dynamic argument. As indicated above, examination of existing dicotyledon familes shows that the more primitive type of floral organisation is associatied with a higher proportion of arboreal types, while the families with advanced floral organisation are wholly or mainly herbaceous. This is surely no coincidence. From the point of view of general evolution the herbaceous type represents a great advance, because its quick reproductive turn-over obviously hastens the testing and resorting of seed-borne variations, whether gene mutations or segregates. The long time-lag of the arboreal type, which may require 10-50 years to reach the reproductive stage, must mean that in a given period of time fewer generations are produced with consequently fewer chances of mutation, hybridisation or segregation. In any given line the arrival of the herbaceous habit might mean a great acceleration of the evolutionary process, provided that the genic equipment of that line contained the necessary potentialities. This aspect of the matter is however outside the scope of the present discussion.

It appears to the writer that arguments from evolutionary trends within the group of the dicotyledons themselves, if such are discernable, are more cogent than arguments from comparative morphology. Turning to the question of anatomical evolution in the dicotyledons, the outstanding feature is the increasing dominance of secondary structures in the stele. This sets the dicotyledons apart from all other existent flora except the gymnosperms, since the vascular cryptogams show only primary stelar structure and the cambial activity of the monocotyledons is negligible.

Before elaborating this proposition further the meaning attached to the terms primary and secondary must be defined. The stele is delimited as procambium by the parenchymatisation of pith and cortex. These are regarded as primary tissues because they are directly derived from the apical meristem: they remain for a time capable of division but do not give rise to cells unlike themselves. The procambium is directly derived from meristem, is capable of division, and such of its units as differentiate directly into xylem or phloem can also be considered primary. Cambium on the other hand cannot be considered a primary tissue. It is not a layer of procambium 'left behind' in differentiation of the vascular segment. The procambial mosaic is irregular, and cambium is characterized by precise periclinal divisions. (The different ways in which cambium becomes established are described in detail in part II.) In addition cambium retains capacity for continued division and its derivatives become elements unlike the formative tissue and are normally incapable of further division.

It seem legitimate therefor to consider that the time of initiation of the vascular cambium is crucial. Further, a stem in which most or all of the vascular tissue is secondary (i.e. is the result of cambial activity) is obviously derivative. This type of development is only found in herbaceous stems and is commonest among the Gamopetalae. Therefor it is possible to conclude on anatomical grounds alone that the arboreal habit is primitive and the herbaceous habit derivative and this conclusion is reinforced by the distribution of these types of stele in relation to systematic position. It might be objected that many herbaceous stems are to be found in families with relatively primitive flowers, e.g. Ranunculaceae. These stems however all show deep procambial strands and a high proportion of primary xylem: cambial activity (where it exists) begins late. One might suggest two lines of herbaceous evolution, one negative, implying general loss of size, diminution of vascular tissue, increase of parenchyma

and meagre cambial activity: the other positive, in which the stelar development is short-circuited and precocious cambial activity takes place. In such cases the result is the production of an effective conductive or vascular system, comparable (as has been pointed out by Sinnott and Bailey (32)) to the first annual ring of an arboreal type, but achieved in a radically different manner. The steles of say Lythrum and Tilia are analogous, not homologous..

The second aspect of the evolution of the dicotyledon stele to be considered is the degree of dispersion of the vascular segments, and here two lines must be followed, one through the predominantly woody families and the other through the predominantly herbaceous. The data given clearly show that in the woody families there is a stabilization of stelar type round the CRO and MRO patterns, indicating that these are the most successful plans for a woody stem. They provide a high proportion of conductive elements while the maintainance of the gap-residues as rays, supplemented by xylem parenchyma and secondary wood-rays, allows ample storage accommodation for surplus carbohydrate, the ever-present problem of the tree.

Magnoliaceae stand alone (except for Proteaceae) in having the stelar pattern CIO. The gap-residue parenchyma in this family averages 10%: many CRO and MRO steles have less than this (see above p. 8). Accordingly it is suggested that the CRO pattern could be derived from CIO by reduction of the gap-residues: this would lead to a further compression of the procambium giving a regular mosaic and so producing the effect of regular primary xylem(see Figs.22,23,55,57.)

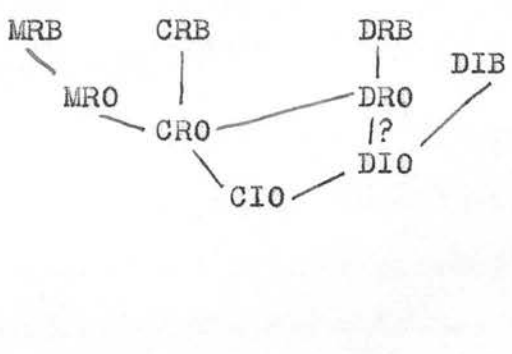
The multisect stele could be derived from the consolidated regular type quite easily. The differentiation of the latter proceeds in such a way that regularly alternating files of xylem and parenchyma are produced.(See Figs. 22,23,55,57.) If the radial parenchymatisation began earlier and proceeded faster than the

xylem differentiation, the procambial arc would be cut up into a number of small strands and the multisect type result. This is in fact the actual course of events, as can be seen in Figs. 77-81. It is in line with the tendency of xylem differentiation to be postponed to suggest that MRO represents an advance on CRO.

In those orders which are mainly herbaceous the dispersed stele predominates, and in the less advanced Polypetalae the commonest pattern is DIO. As CRO could be derived from CIO by consolidation, so could DIO be derived by dispersion (increasing the size of the gap-residues).

In the higher Polypetalae and the Gamopetalae the pattern DRB makes its appearance. The evolutionary significance of this pattern has already been discussed. It is frequently associated with precocious cambium. Generally speaking the more dispersed the vascular segments the greater the likelihood of finding precocious cambium, but this is not a universal rule. For example, Lamium with highly dispersed stele (G.R.P. more than 60%) has a precocious cambium, and so does Veronica (G.R.P. less than 10%). In these bound steles the joining segments which bind together the original vascular bundles are mainly vascular or vascular and mechanical: the diminution in importance of the 'rays' and other storage tissue can be correlated with the different carbohydrate balance sheet of the herb in contrast to that of the tree.

From the purely structural point of view the possible relations between the various stelar patterns may be indicated in the following scheme:-



In regard to the condition of the leaf-gap, it will be seen from the data that the trilacunar gap may be associated with every kind of stelar pattern. The multilacunar gap is usually found with large or sheathing leaves and moderately dispersed steles. The unilacunar gap is more frequent in the gamopetalae, but not associated with a particular stelar type. It will be seen that no precise corelations can be indicated between gap-type and stelar type.

Evolutionary trends within the group of the dicotyledons may accordingly be summed up as follows:-

I). Increasing dominance of secondary structures in the stele:

associated with - progressively earlier initiation of vascular cambium, decrease in amount of primary xylem:

culminating in - supersession of primary by secondary xylem.

2). Increasing size of gap-residues:

associated with - increased dispersion of vascular segments and their relative diminution in size:

culminating in - extremely dispersed herbaceous stem.

On anatomical and systematic grounds it is concluded that the consolidated irregular open stele is the most primitive: that the consolidated regular open stele and the multisect stele are the most efficient for the arboreal type of stem: that the most advanced type of structure is the extremely dispersed herbaceous stem with precocious cambium and a bound secondary stele.

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PART II. ONTOGENY OF THE STELE.

I. THE SHOOT APEX IN THE ANGIOSPERMS.

The present position of our knowledge of the organisation and behaviour of the shoot apex in the angiosperms has recently been fully reviewed by Foster (I4) where a comprehensive bibliography will be found. Only the leading issues will be considered here.

Foster points out that study of the apex was for long dominated by the conception of apical initials, carried over from earlier pteridophyte studies. This was superceded by Hanstein's (I5) conception of histogenic layers - 'dermatogen' giving rise to epidermal system: 'periblem' giving rise to cortex and inner leaf-tissues: 'plerome' giving rise to conducting system and pith. Recent histogenic studies have shown that this rigid connexion is by no means universal in angiosperms.

The most recent interpretation of the structure of the shoot apex is based upon the 'tunica-corpus' conception, first formulated by Schmidt (28) and extensively developed by many workers. (For full references see Foster (I4)).

Schmidt recognized only two tissue zones at the apex:-

I). The 'tunica' of one to many layers, peripheral in position and characterised by surface growth and anticlinal divisions.

2). The 'corpus', a central tissue characterized by volume growth, irregular arrangement of cells and divisions in all planes. There is considerable variation in the number of layers belonging to the tunica and in the degree of contribution of the corpus to the leaf-primordia.

Foster points out that this theory has the great value of focussing attention on the dynamic aspect of apical growth, " in that the demarcation of zones reflects the distribution of well-coordinated growth patterns which may be expected to fluctuate within wide limits in the various groups of angiosperms."

Louis (20) has dealt fully with the constant growth adjustments at the shoot apex, showing the regular succession of phases of maximal and minimal radial expansion of the shoot and their relation to the production of the 'soubassements foliaires' upon which the leaf primordia develope. Louis' work is fully documented and so clearly presented that it is indispensable to workers in this field.

In a series of papers on experiments in phyllotaxis (35,36, 37) M.Snow and R.Snow emphasise the importance of considering the shoot apex as a whole. They show that the placing of new primordia depends upon the available space left for them, which depends in turn upon the relations of the other older members which are still within the apical zone.

It is unfortunate that preoccupation with earlier work on the vascular cryptogams should have influenced the study of the angiosperm apex so strongly. Even quite recent work refers to presumed initials, but on examination of the evidence it is not convincing. It appears to the writer to be quite unnecessary to expect apical initials in the angiosperms. The massive apical cone with its rhythmic growth-thrusts which produce 'soubassements' its regular sequence of lateral apices produced in relation to leaf primordia, is far in advance to any pteridophyte and of most gymnosperms in plasticity. A.H. Church remarked (in litt.) that " the angiosperm apex had freed itself from the tyranny of a single apical cell." Why then should it return to the modified bondage of a 'group of initials' or even three rigid histogenic layers? Church interpreted the phenomena of phyllotaxis in terms of space-relations of primordia and balance of the whole shoot against gravitation. His mathematical treatment of the subject will be found in (7,8).

This general growth-balance must obviously be taken into account, but modern work on growth-hormones suggests a further controlling and integrative factor.

In the following discussion the term auxin will be used in a general and not a specific sense, as meaning any growth-hormone without reference to its chemical nature.

Certain facts about the location, movement and effects of auxins are now well established.

I). Auxin is produced in the apical regions of shoots and coleoptiles: the more terminal the position of the bud, the greater the concentration of auxin. (Skoog(33)).

2). Light (Went (43),Boysen-Jensen (6)) and gravity (Navez and Robinson (22)) induce unequal distribution of auxin: auxin migrates to the shaded side of organs in unilateral illumination and to the lower side of horizontally placed organs, the resulting curvatures being proportional to the concentration of auxin.

3). Nastic movements result from unequal distribution of auxin. (Zimmerman and Wilcoxon(49) and Avery (I)).

4). Transport of auxin is basipetal. (Went (43)).

5). Auxin controls cell enlargement.(Went (43),Boysen-Jensen (6)).

To these must be added the suggestion that wall-growth of the cell is the cause and not the consequence of vacuolation. This is supported by the findings of Ursprung and Blum (42) that the diffusion pressure deficit (osmotic pressure) of dividing cells is high but their turgor pressure low. Continued areal extension of the wall would tend to keep the wall pressure and therefor the turgor pressure low.

In attempting to correlate these facts with events taking place at the shoot apex, it may be pointed out that:-

I). In its earliest stages the leaf primordium is growing faster than the shoot apex.

2). In its earliest stages the leaf primordium shows hyponastic growth.

3). When the leaf begins to unfold it shows epinastic growth.

This suggest that, in its earliest stages, the leaf primordium is 'more terminal' in position than the shoot apex, and is

therefor producing more auxin. Moreover, the hyponastic growth indicates a greater production of auxin on the abaxial side of the primordium. This should lead to a more rapid cell-enlargement and vacuolation on the abaxial side of the primordium and (by basipetal transport of auxin) in the 'soubassement' and the sector of axis immediately underlying it. Inspection of Louis' (20) figures show that parenchymatisation does in fact begin on the abaxial (or as he calls it ,dorsal,) side of the leaf primordium. This is confirmed by the writer in numerous cases.

When the leaf begins to unfold it enters upon another phase of rapid growth, this time epinastic, with corresponding increase of auxin production on the adaxial side. This might be expected to cause increased parenchymatisation on the adaxial side of the leaf trace strand: this has been found to be the case. It was suggested above (in the discussion of the multisect condition) that the location and extent of the interfascicular parenchyma depended upon the relative rates of xylem differentiation and parenchymatisation. If this is so, it should be possible to correlate the stage of differentiation of the stele with the development of the leaf. There is in fact a strong correlation between the two. This is not presented as a new idea, because it has already been noted by other workers (e.g. Pristley(25)), but the further connexion with auxin supply is a novel one. This connexion can be extended to cambial growth, as shown by Snow (38) but as this is a secondary stage it will not be considered here.

There remains the question of the vacuolation of the pith, which usually precedes that of the 'soubassement'. It is suggested that this is the result of auxin production by the apical meristem itself, because it appears at a time when the 'soubassement' is newly formed and growth of the leaf primordium has scarcely begun.

These suggestions about auxin and differentiation do not of course explain the integration of the different growth phases, which is the ultimate mystery of plant growth. Nor do they touch upon the question of the existence of specific 'organisers' analto those known in the animal kingdom. In this connexion the observations of Williams (44) upon the effect of heteroauxin on Selaginella are significant. He found that the application of heteroauxin to the angle-meristems of Selaginella induced the formation of rhizophores. Much experimental work has also been done on root induction (e.g. Zimmerman,Crocker and Hitchcock(47), Zimmerman and Hitchcock (48), Zimmerman and Wilcoxon (49)), but the substances used - carbon monoxide, unsaturated hydrocarbons, substitute fatty acids - are so foreign to ordinary plant metabolism that it is difficult to translate the results into the terms of normal morphology and development. There is here a wide field for cooperation between the biochemist, morphologist and anatomist, but in the present state of knowledge one is forced reluctantly to leave these major questions unanswered.

2). THE APICAL MERISTEM: PROCAMBIUM.

Zirkle (5I) has shown conclusively that no studies of the cytological condition of cells at the apical meristem are valid if the older nuclear fixatives alone are used. In his paper "Vacuoles in Primary Meristems" he examined the apices of the following angiosperms: Robinia, Phaseolus, Polygonum, Fraxinus, Zea. Comparing fixed and living material, he confirmed the existence of normally spherical vacuoles in all cells of the apical meristem. He also stated that in procambial cells the vacuoles are smaller and usually fixed as canals. (This may be connected with the change of shape of thess cells under compression by surrounding tissue.)

Priestley denies the presence of vacuoles in meristems. He describes the cell-types of growing points as follows:-

I). meristematic, 2). vacuolating dividing,3).vacuolating extending. In "The Meristematic Tissues of the Plant" (23) he states "neither plastids nor vacuoles are reported as present in this cytoplasm." A few lines further on he says " from the cytological standpoint the cytoplasm of the meristem cell in fixed and stained preparations might often be described as interspersed with very fine vacuoles", but he does not consider them as the equivalent of the vacuoles " charged with vacuolar sap containing solutes exerting osmotic pressure" seen in more differentiated cells. This paper is a review, but he repeats the same ideas in "Cell growth and cell division in the shoot of the flowering plant" (24). There he says " in properly fixed material" the meristematic cell appears as a small cell with dense protoplasmic contents and prominent nucleus: " no vacuole is recognizable". A photograph of the L.S. of the apex of Syringa vulgaris is given, but no details of fixation. His work antedates that of Zirkle.

Bailey's (3) extensive studies on cambium also show the existence of vacuoles in this tissue.

The writer, using Zirkle's technique, has confirmed vacuolation of apical cells in over 70 species (see list below).

It seems therefor that our conceptions of the course of differantiation at the apex require to be modified. In place of an unvacuolated cell, which by some change of metabolism gains the power to take in water with consequent vacuolation and enlargement of the cell, we start with a protoplast already vacuolated. Under the influence of auxin the areal growth of the wall is followed by a distention of the protoplast: existing vacuoles are stretched, merge, and finally give the large central vacuole of the parenchyma cell.

Older work on the development of the stele had already indicated the relatively late differentiation of the procambium. It may be that this region lags in comparison with the pith and cortex

because of its position: it is not in the direct stream of auxin transport. It is generally accepted that the cells of the procambium assume their characteristic elongated shape as a result of the pressure exerted on both sides by the earlier vacuolating pith and cortex.

Kostychev (I8) showed that the procambium ring is, in its earliest stages, continuous in the majority of dicotyledons. This appears to correspond with Louis' prodesmogen stage. Louis' results show with great clarity that, by the coordinated parenchymatisation in successive 'soubassements', the stelar plan is marked out in the procambium stage, the full stelar pattern however not being seen until after the development of a number of leaf-gaps, depending upon the phyllotaxis in the individual case. As a result the procambial ring is divided into a number of segments (leaf trace segments) separated by parenchymatous segments of varying width. It is obvious that these parenchymatous segments are part of the leaf-gap system: they should (as pointed out above) be called gap-residues. They are commonly referred to as 'primary rays': this obscures their relationship to the whole stelar plan and should be abandoned. Later, they may function as 'rays' and may undergo histological modification to that end, but such a development is secondary to the main theme of progress of the ontogeny of the stele.

3). DIFFERENTIATION OF THE STELE.

The further development of vascular tissues from the procambium stage has been but little studied, and accounts given in the literature are extremely vague (e.g. Eames and McDaniels (II)). Louis (20) mentions the appearance of the first lignified elements in some cases, but does not go into detail. Kostychev (I8) has presented some studies of the procambium and early xylem differentiation in dicotyledons, but he does not give exact evidence of the origin of procambium from apical meristem and his illustrations of the further development are meagre. His reagents were Eau de Javelle and NaOH, which do not inspire confidence in his results.

Thoday in his valuable paper "On the organisation of growth and differentiation in the stem of the Sunflower" (41) gives full details of the procambial stage and the initiation of periclinal divisions on the inner margin of the procambial crescent, pointing out that the whole of the xylem is formed from this cambium. He states also that the procambial strand itself increases in size by cell division. Phloem differentiation begins in the centre of the strand and proceeds laterally until complete. In modern terminology, the phloem of Helianthus is primary and the xylem wholly secondary.

Priestley in his review "The Meristematic Tissues of the Plant" (23) mentions (in connexion with the origin of cambium) that xylem differentiation on the inner side and phloem differentiation on the outer side of the procambium occur almost contemporaneously with the characteristic tangential cambial divisions, although he also suggests that the latter activity may precede the former. In a later study of the vascular anatomy of Helianthus annuus (25) he makes the following statements. Vacuolation is first seen in the sixth primordium from the apex, isolating a distinct procambium strand: the first primary protophloem cell is also seen. "Tangential longitudinal divisions indicative of cambial activity were first evident at the base of the seventh primordium. Here also the most adaxial of the radially seriated cells showed a slightly larger size and less dense contents: comparison with older primordia made it clear that these were the earliest signs of the differentiation of secondary protoxylem in the central strand. Both procambial origin of protophloem and radial seriation, and hence cambial origin, of the protoxtlem, were described by Thoday. Lehmberg also noted the radial seriation of the protoxylem. This sequence of appearance of primary protophloem, cambium, secondary protoxylem appears to be very general." No figures of this stage of development are given in this paper, but in his book "An Introduction to Botany" (26) p.299, fig.73, a few cells from T.S. of Privet are shown.

The exact orientation of the drawings is not given, and the scale and detail are too small to be convincing. Lehmberg's (19)drawings are extremely meagre: he indicates roughly the xylem only without surrounding tissues.

Priestley's generalisation about the sequence of tissue-differentiation appears to be too sweeping. The writer has examined the apices of a number of types covering all sections of the dicotyledons and including woody as well as herbaceous forms, and has come to the conclusion (as indicated previously) that this precocious cambium is by no means universal: it is in fact considered as the climax of evolution in the dicotyledon stele.

It is obvious that the time and mode of origin of the vascular cambium is of fundamental importance. As already indicated, two distinct modes of differentiation of primary xylem have been recognised in the dicotyledons. Detailed evidence of differentiation of xylem and origin of cambium in selected types illustrating these modes will be presented below.

3.DIFFERENTIATION OF THE STELE: ii). Material and technique.

The following is a list of the material examined. Apices were fixed in Zirkle and Bouin (see below) at the time of most rapid growth.

Acer pseodoplatanus (Aceraceae), Aesculus hippocastanum (Sapindaceae), Anamirta cocculus (Menispermaceae), Apodytes dimidiata (Icacinaceae), Ardisia esculenta (Myrsinaceae), Betula alba (Betulaceae), Buddleia variabilis (Loganiaceae), Bursera cuneata (Burseraceae), Capparis inermis (Capparidaceae), Cassine maurocenia (Celastraceae), Ceropegia Woodii (Asclepiadaceae), Chironia sp. (Gentianaceae), Chrysosplenium oppositifolium (Saxifragaceae), Clusia sp. (Guttiferae), Coleus Blumei var. (Labiatae), Columnea vedrariensis (Gesneraceae), Cornus sanguinea (Cornaceae), Daphne mezereon (Thymeliaceae), Deherainea smaradgina (Myrsinaceae), Delphinium, var. hort. (Ranunculaceae), Desfontainea spinosa (Loganiaceae), Diospyros ebenum (Ebenaceae Elaeocarpus obovatus (Tiliaceae), Empetrum nigrum (Empetraceae) Epilobium montanum (Onagraceae), Erica carnea (Ericaceae), Erythroxylum coca (Erythroxylaceae), Escallonia sp. (Saxifragaceae) Eugenia jambo (Myrtaceae), Euonymus japonica (Celastraceae), Frankenia hirsuta (Frankeniaceae), Galium aparine (Rubiaceae), Goodenia ovata (Goodeniaceae), Grewia parvifolia (Tiliaceae), Ilex aquifolium (Aquifoliaceae), Illycium floridanum (Magnoliaceae), Fraxinus excelsior (Oleaceae), Hypericum sp. (Hypericaceae), Jasminum nudiflorum (Oleaceae), Ledum palustre (Ericaceae), Linaria purpurea (Scrophulariaceae), Liriodendron tulipiferum (Magnoliaceae), Moringa oleifera (Moringaceae), Murraya exotica (Rutaceae), Myoporum laetum (Myoporineae), Myrsine africana (Myrsinaceae), Nepeta hederacea (Labiatae), Persea sp. (Laurineae), Peumus boldus (Monimiaceae), Physalis peruviana (Solanaceae), Platanus sp. (Platanaceae), Platytheca galioides (Tremandraceae), Plumbago capensis (Plumbaginaceae), Polygala myrtifolia (Polygalaceae), Psidium montanum (Myrtaceae), Reintwardia tetragyna (Linaceae), Rhododendron praecox (Ericaceae), Rhus nervosa (Anacardiaceae), Ruellia sp. (Acanthaceae), Salix sp. (Salicaceae), Sambucus nigra (Caprifoliaceae), Samyda obliquata (Samydaceae), Santalum album (Santalaceae), Serjania cuspidata (Sapindaceae), Shorea robusta (Dipterocarpaceae), Skimmia japonica (Rutaceae), Steriphoma elliptica (Capparidaceae), Stuartia sp. (Theaceae), Styrax Wilsoni (Styracaceae), Syringa vulgaris (Oleaceae), Thea sinensis (Theaceae), Ulmus montana (Ulmaceae), Vallaea stipularis (Tiliaceae), Veronica agrestis, V. amethystina (Scrophulariaceae), Vinca minor (Apocynaceae).

In order to study the early stages in the differentiation of the stele, it was necessary to find a satisfactory cytoplasmic fixative which would give a reliable image of the stages in vacuolation. Earlier workers in this field have employed general or nuclear fixatives, such as formalin alcohol, chrom-acetic or picro-formal mixtures. None of these give adequate cytoplasmic fixation and the results obtained cannot be considered altogether valid.

After considerable experiment Zirkle's (46) fixative for vacuoles was chosen for all detailed work. The formula is:-

Chromic sulphate $Cr_2(SO_4)_3.15\ H\ O$....	5.0 grams
Copper oxide CuO.....	0.5 grams
40% Formaldehyde.....	10.0 cc.
Water.....	90.0 cc.

Zirkle has stated (47) that the image obtained after fixation with this mixture agrees well with observations on living cells at stem and root apex: this has been confirmed by the writer. Material was fixed 24 hours in the above.

Duplicate lots were also fixed in Bouin's Fluid:- (5).

40% Formaldehyde.....	25cc.
Acetic acid, pure glacial.....	5cc.
Picric acid, saturated aqueous.....	75cc.

This was the fixative recommended by Louis(20). It gives satisfactory results for the actual apical cone, but less so for the stages after rapid vacuolation has set in. The nuclei are clearer than in Zirkle, the nuclear membrane and nucleoli being particularly sharp. It forms a useful check on the Zirkle material and having a greater power of penetration than the latter it can be used for slightly older stages. Moreover it has the great advantage that material may be left in it almost indefinitely.

Stems examined in the mature state only were either cut fresh by hand or after fixation in strong formalin-acetic alcohol (absolute alcohol 6, 40% formaldehyde 3, glacial acetic acid 1).

Instead of fixation at the time of gathering, as is usually advocated, it was found that superior results were obtained by cutting the stems a few inches from the apex and placing them for some hours in a large vessel of water in a cool place. This was specially important when dealing with hot-house material, which is usually slightly wilted even in the morning. By getting the material into a state of full turgescence, the fixatives appeared to be able to penetrate more easily: one or two minutes under a gentle vacuum being enough to secure sinking.

Both the fixatives used softened the material, so that a simple and effective method of handling through the alcohols was devised. Small tubes were made of fine cotton net. These were closed at one end and held open by two bone curtain rings stitched into position, one at eahh end of the tube. After fixation the material was put into these tubes and half-a -dozen tubes placed in a 250cc. straight-sided tap funnel. The required fluid was gently added from above, and after the required time run off from below. In this way large batches of material could be handled in a short time and with the minimum of manipulation.

Grades of alcohol differing by 5% were used up to 90%, 2 hours in each. Then 2 hours in each of absolute alcohol, equal parts of absolute and cedar oil, and finally pure cedar oil. (Material can if necessary be left indefinitely in the pure oil). When required for embedding it was transferred to a closed capsule containing equal parts of cedar oil, benzene and paraffin, at oven temperature. Then two changes of pure paraffin, one hour in each. The wax used was Gurr's filtered extra pure, M.P.52°C., modified by slight overheating and the addition of about 5% beeswax. The wax was remelted and used repeatedly. This gave a beautiful clear glassy matrix with not tendency to crystallise. Except where otherwise stated all sections were cut at 10μ.

The only stain used in the differentiation studies was Mallory's Ferric chloride - Haematoxylin (McClung (21)).

The sections are mordanted for 5-10 minutes in 10% Ferric chloride, thoroughly rinsed and stained 5-10 minutes in 1% Haematoxylin (both solutions aqueous). After staining, differentiate in 0.25% Ferric chloride. As the local water was very soft, the preparations were washed after differentiation in water brought to pH.8.4 by sodium carbonate. This gave a beautiful blue tone, very satisfactory for oil immersion work. Mounting was done in Gurr's Neutral Medium.

Occasional use was made of Erythrosin and Light Green in counterstaining for protoxylem and protophloem respectively: the usual I% solutions were used.

For purely anatomical work on mature stems Safranin and Aniline Blue were used.

Recording. Low-power drawings and outlines were made with a microprojector. For high-power work a camera lucida was used.

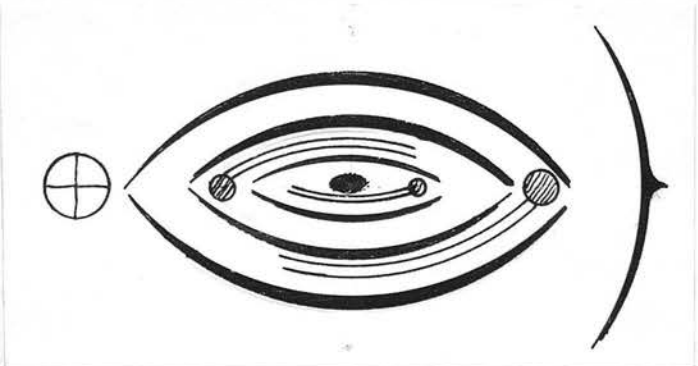
Optical equipment. For low-power work, Watson's parachromatic series 2", 2/3" objectives were used. For high-power, Reichert's apochromatic objectives 4mm and 2mm.(N.A. I.30) were used with holoscopic eyepieces x6, I2 and I8, and Watson's Universal Condenser. Critical illumination was secured by the use of a naked-filament low-voltage lamp, which, in conjunction with a blue-green screen (Wratten no. 44) gave maximum resolution for oil-immersion work.

3.DIFFERENTIATION OF THE STELE.iii). Detailed presentation of the evidence.

Type I. LIRIODENDRON. Stelar pattern CIO.

Liriodendron tulipiferum is a tree type with petiolate leaves in distichous phyllotaxis and a multilacunar leaf gap.

The series studied was taken from a bud just beginning to open. The bud-scales are stipules. The bud is very flat and stands edge-on to its subtending leaf. At this stage the two rounded-oblong outer stipules enclosed three formed leaves and four stipules visible to the naked eye. Serial sections indicated the presence of another newly developed leaf with its stipules forming a complete 'hood' over the bud-apex. The leaves of Liriodendron are peculiar in shape and in their placing in the bud. They are folded over on their midribs and, the petioles being relatively long at this stage, each leaf is bent over with its tip downwards, the blade margins facing the petiole. Each leaf in turn lies to one side of the enclosing stipules of the next leaf, right and left of the bud-axis in turn. The following diagram illustrates this, as does Fig. 29 of outlines drawn from serial sections.



As seen in transverse section the stem apex is a flattened oval. Vacuolation appears in the pith after one plastochrone (distance from apex 80μ). The cortex is visible after II0μ, and the stele is marked out as a ring of small procambial strands after two plastochrones (I50μ). Although this stage is reached fairly quickly, further differentiation of the stele is slow. No signs of vascular elements were visible until just above the level of insertion of the third leaf (distance 440μ). A slightly later

stage (after three plastochrones, distance 470μ) is illustrated in Figure 30, which shows the small procambial strand surrounded by parenchyma. Three xylem elements are seen: the phloem is organised but no actual differentiation has taken place and there are no regular periclinal divisions. Further development proceeds as in Magnolia (Fig.2) and the secondary stele is similar in pattern (Fig.I).

The complete encirclement of the stem by the stipules and the numerous strands contributed at each node lead to the presence in the stelar ring of bundles of different size, age and state of differentiation. Serial transverse sections of the bud indicate that, instead of localised 'soubassements', this apex produces a ring-shaped structure suggestive of the sheathing leaf-primordium so common in the monocotyledons.

The large pith is reminiscent of a herbaceous stem, and in a sense the primary stage of Liriodendron could be described as 'many-bundled.'(See Clematis,Fig.II, Delphinium,Fig.3I). The vascular segments however are close-set in Liriodendron (G.R.P. 10%) while in Delphinium they are widely separated. Nevertheless the irregular mode of xylem differentiation is the same and emphasises their systematic connexion. It was suggested above that the Magnoliaceous stem could be 'transformed' into the Ranunculaceous by increased dispersion (increase in proportion of gap-residue parenchyma): a comparison of Figs. I,II and 3I bears this out.

The stems of Magnoliaceae are unique in their combination of a consolidated pattern with irregular primary xylem, open secondary stele and divergent histology of the mature xylem.

Type 2. DELPHINIUM. Stelar pattern DIO.

Delphinium is a herbaceous perennial with a stout aerial stem and large compound leaves, spirally arranged. The pith is large and often disintegrates in the lower internodes leaving a hollow stem.

The material used was a garden form. In order to avoid flowering axes the massive buds were taken as soon as they appeared above ground and became green. To ensure satisfactory fixation very short pieces were taken, but these proved sufficient to include the desired stages as differentiation is rapid.

Longitudinal sections show the stem apex to be rather large and hemispherical in form, the soubassements being produced well down on the flanks of the apical cone. Very rapid radial expansion takes place, mainly in the pith, so that the whole section shows the apical meristem as a hemisphere poised upon a much larger hemisphere. (See Fig. 32). The tunica is a single layer only.

In serial transverse sections the regularity of the spiral phyllotaxis is well shown. The leaf-primordia show the effect of close-packing: those of the third cycle from the apex are rhomboidal in section while the outer free members are triangular(Fig.3I)

Three leaf-primordia join the axis within 60μ from the apex. The next cycle of three have joined within I40μ, the third cycle within 230μ, the fourth within 370μ and the fifth within 790μ. (I.e. 60,80,90,I40,420μ between cycles). That is, internodal extension scarcely begins until between the third and fourth cycles of leaves. Division by vertical walls begins in the pith immediately behind the apical meristem and the marked radial expansion of these cells is seen about 400$\mu$from the apex, about the level of insertion of the fourth cycle of leaves. This expansion causes the pith cells to separate in horizontal 'strings' with intercellular spaces between the layers and gives the characteristic loose airfilled pith of the older stem.

The stele is marked out as procambium after the insertion of the fifth leaf (I30 μ from apex), the individual strands being separated by parenchyma. It has been stated by Kostychev (I8) that the procambial stage in Ranunculaceae take the form of separate strands from the beginning. This is not the case in Delphinium: a continuous prodesmogen ring is seen at the level of insertion of leaves I-3 which come on practically at the same level. The crowing of the primordia and the rapidity with which this stage passes over to the next of separate strands suggests that the prodesmogen ring was missed by Kostychev. (See Fig. 33).

The first protoxylem element is seen in the median trace of leaf 7, I80 μ from apex (Fig. 34). Protophloem appears in the next leaf (leaf 8) I90 μ from apex, Fig. 35. At the level of insertion of leaf II the bundle is well organised. Several phloem elements have appeared in the medial trace of leaf II, there are about eight primary xylem elements scattered amongst the xylem parenchyma and periclinal divisions are in evidence across the centre of the strand Some secondary xylem in radial seriation has been formed. On the cortical side of the bundle are seen the beginnings of the cap of fibres which is a feature of the mature bundle (Fig. 36). The number of cells in the bundle at this stage shows that the procambium increases by division before differentiation is complete: this is also suggested by the strained condition of the parenchyma between the bundles.

Delphinium is a typical example of the mode of differentiation which results in the stelar pattern DIO. Other examples of irregular xylem differentiation coupled with both consolidated and dissteles are given in Liriodendron (above), Sambucus, and Plumbago (below).

Type 3. TILIA VULGARIS. Stelar pattern CRX'O.

The stele of Tilia was chosen as representative of the consolidated regular pattern.

The annual increment of the shoot of Tilia is monopodial, but the shoot-system as a whole is sympodial owing to the death of the terminal bud of each year's growth. The number of leaves on one year's growth varies but the average is nine. The phyllotaxis is distichous. The relation of the axillary buds to the leaf-bases and of the leaves to the whole spray has already been described and figured by the writer. (34).

The resting bud is completely covered by two overlapping bud-scales and is flattened on its adaxial side as compared to its abaxial side. The outer bud-scale completely overlaps the inner and is fused with the stem for a much greater distance above its insertion. It is not proposed to discuss the morphology of these scales here, but their vascular supply suggests that they are equivalent to leaves stipules.

In the resting bud we are dealing with a temporarily static phase: growth has come to a standstill and no more members are being added to the system. Serial sections of resting buds taken in June show that the bud at this stage consists of two bud-scales, the bud-axis and four leaves with their stipules. A bud dissected in March showed the stipules (which function as inner bud-scales) fully developed while the associated laminae were only about 2mm. in length. There are no signs of buds of the second degree in the resting bud, except for a minute incipient bud-meristem in connexion with the inner bud-scale. (See Figs. 37, 38). In fully expanded shoots of the current year this bud can be seen subtended by the bud-scale scar: it can be made to develope by removing the shoot above it.

The exact and beautiful symmetry of the phyllotaxis system is well seen in both the resting and the opening bud. It is evident that a strict distichy rules the development of the leaf members with their associated buds and stipules. The centres of the petioles lie on a straight line, the long axis of symmetry of the bud. The axis of symmetry of the bud is not however at right angles to that of the main stem (see Figs.37,38). A similar symmetry appears in the relations of the buds of the second degree(Figs.39-42).

The stipules on the adaxial side of the bud-system are larger than those on the abaxial side and in the dormant bud they markedly overlap the abaxial members.

For convenience in comparison the parts have been numbered from the apex downwards in both series. The whole series in the opening bud included five leaves and their stipules,the lower stipules having been trimmed off for fixing.

The order of junction of parts in the resting bud is as follows.

Joining member:	distance from apex, μ.
Stem apex -	-
adaxial stipule I	20
petiole I	40
adaxial stipule 2	60
abaxial stipule I	80
adaxial stipule 3	I60
petiole 2	220
abaxial stipule 2	280
petiole 3	280
adaxial stipule 4	380
abaxial stipule 3	400
inner 'bud-scale'	5I0
petiole 4	540
abaxial stipule 4	600
outer 'bud-scale'	840
(subtending petiole)	890-I000

It will be seen that several adaxial members join the axis in sequence. It appears that this is question of space in the resting bud because in the opening bud there is a definite rhythm and succession of adaxial and abaxial fusions as will be seen. The resting bud is shown in Figs. 37,38.

The order of junction of parts in the opening bud is as follows.

BUD SHOOT joining member:	LATERAL BUDS:	joining member:	distance from main apex: μ.
stem apex			
adaxial stipule I	apex 2		50
petiole I	apex 2	adaxial stipule 2	80
abaxial stipule I	apex 2	petiole 2	90
bud 2+ petiole 2+ adaxial stipule 2			I00
abaxial stipule 2	apex 3		I60
	apex 3	adaxial stipule 3'	
	apex 3	abaxial stipule 3' adaxial stipule 3	
bud 3+ adaxial stipule 3			340
petiole 3			380
abaxial stipule 3			4I0
bud 4+ petiole 4			970

The sequence of joining of lateral bud 5 and petiole 5 are similar: bud 5 has two pairs of stipules and the suggestion of a bud-meristem (of the third degree) upon it.

It will be seen that the whole of next season's growth is not preformed in the resting bud: the emergent bud-axis is capable of continued growth. Comparing the resting and expanding buds it will be noted that buds of the second degree are not seen in the resting but, except for the slight indication of a meristem in connexion with the inner bud-scale. In the expanding system several secondary buds are to be seen. Moreover stelar differentiation in the resting bud has not proceeded beyond the procambial stage even at the base of the shoot(i.e. at the level of the fourth leaf from the apex.) In the expanding bud considerable differentiation of the stele can be seen at a corresponding level. This will now be described.

Differentiation of the stele. As seen in longitudinal section the stem-apex of Tilia is rather flattened. It is not in strict vertical alignment with the axis below, but appears to diverge away from the direction of the youngest soubassement. The tunica consists of about 4 layers of deeply staining cells, which Zirkle fixation shows to be lightly and evenly vacuolated. Bouin fixation reveals numerous nuclear divisions at this level(Figs.43,44).

Serial transverse sections show that the pith is marked out

by increasing vacuolation after one plastochrone, and after two plastochrones the cortex is also clearly seen, thus delimiting the stele. At this stage the whole stele is procambial, deeply staining and showing prominent nuclei. (Figs. 45,46). The orientation of the cells is the usual irregular procambial mosaic.

After three plastochrones the first lignified elements are visible in the median leaf-trace segment of the third leaf (Fig.47). These first-formed xylem elements are derived from procambial cells: their origin can be traced cell by cell from the apex. It therefor appears legitimate to describe them as primary. They occur in groups of two or three, separated laterally by cells which are deeply staining, lightly vacuolated and possessing prominent nuclei. These latter cells are destined to become files of parenchyma separating the files of xylem in the mature stele.

Lower down in the same internode the first signs of secondary development are seen, namely the appearance of periclinal divisions opposite the differentiating xylem only. (See Fig.48).

Meanwhile the files of parenchyma have been keeping pace with the increasing radial depth of the xylem by radial enlargement, accompanied by one or two anticlinal divisions. As a result the files of parenchyma appear to wedge apart the developing xylem. Concurrently with these changes fully vacuolated cells at the outer margin of the pith and (as will be proved later) in the pericycle, become filled with large confluent drops of some deeply staining substance. These cells are different from the well-known mucilage sacs, whichappear later: the nature of the stainable substance has not yet been determined. These cells follow closely the outline of the developing stele, later on marking out the phloem segments very clearly.

Not until after four plastochrones do periclinal divisions appear in the files of parenchyma. The first division to be noted in this situation is shown in Fig.49.

It will be seen that the cambium in the vascular segments of this type is not cut out as a whole from the procambium: it is, even at this early stage of differentiation, heterogeneous. Periclinal divisions originate opposite the xylem only and the establishment of regular cambial divisions across the whole segment is not complete until the fifth plastochrone has elapsed.

The state of vacuolation of the intrafascicular parenchyma must now be considered. Up to a point, as stated above, this parenchyma keeps pace with the xylem by cell-enlargement only. At the time of initiation of periclinal divisions in these cells they are not fully vacuolated, in the sense that the medullary and cortical parenchyma is vacuolated. The intrafascicular parenchyma cells possess very evident vacuoles, but these have not fused to give one large central vacuole as in the cells of the pith and cortex. The nuclei moreover are still central in position, being prominent and highly stainable in Bouin material. It is obvious that these cells can quickly resume rapid and regular nuclear divisions.

There remain the gap-residues which must be spanned before the closure of the cambium ring is complete. The gap-residue parenchyma is fully vacuolated, so that the resumption of active division is a different problem. There is little cytological evidence on the origin of interfascicular cambium. The present type proved unfavourable for its study, but evidence will be given below from other types. Only the time of closure of the ring in Tilia will be described here.

In a young shoot taken at the time of most active growth the gap-residues were not closed until the ninth internode from the apex was reached. The feature which made it difficult to study the details of the process was the presence of the mucilage sacs. They were extraordinarily prominent at this active stage and by their distortion of surrounding tissue and their

avidity for stains obscured fine detail. Once the cambium ring is complete secondary growth proceeds smoothly and rapidly and the familiar one-year stem of Tilia is produced.

One other point deserves mention: the presence in the young stem of a starch sheath. This does not appear to have been recorded. Material taken in August when extension growth was complete or nearly so, showed a very well defined starch-sheath in the third internode. In the eighth internode of the same shoot the starch-sheath was strained, distorted and ruptured, but still detectable. Further down it was quite obscured by secondary growth of the stele with consequent pressure. Although so well defined by its contents, no treatment revealed a casparian strip in this sheath. In the opening bud no starch could be seen except in the lowest internode(below leaf 5) but the large clear cells of the sheath could be easily made out. The outer layer of cells with stainable contents mentioned above lies in immediate contact with the starch-sheath: it is therefor clear that they belong to the pericycle.

Type 4. ULMUS CAMPESTRIS. Stelar pattern CRX'0.

The phyllotaxis of Ulmus is distichous. In the very young stem the axis is not straight but zig-zag, the apex being strongly deflected away from the last formed leaf. As growth proceeds the stem straightens out except at the tip.

The resting bud is enclosed in overlapping bud-scales which consist of fused pairs of stipules and usually includes four leaves with their stipules and the bud apex. The leaves in the bud are folded over on their midribs, the edges of the blades being turned towards the subtending leaf.

The series studied was taken from an opening bud and included seven leaves with their stipules, the lower scales being trimmed off for fixing.

The long axis of the bud is at right angles to the main stem: the stipules are very prominent at this stage. The leaves join the abaxial side of the bud-axis, having first fused with the abaxial stipule. (Figs. 50, 5I).

The following is the order of junction of parts:-

a, adaxial stipule: b, abaxial stipule: L,leaf.

Joining member:	Plastochrones:	Distance from apex: μ.
Stem apex	-	-
b'	I	30
bI + LI	2	50
b2 + L2	3	70
b3 + L3	4	I00
aI	5	I20
a2	6	I40
b4 + L4	7	I60
a3	8	270
a4	9	290
b5 + L5	I0	300
b6 + L6 (bud 5)	II	470
a5	I2	500

Joining member:	Plastochrones:	Distance from apex: μ.
(bud 6)		540
a6	I3	590
b7+ L7	I4	620
a7	I5	720

The course of vascular differentiation is essentially the same as in Tilia: protoxylem appears first and periclinal divisions originate opposite the xylem only.

The apex is meristematic for a depth of about 50μ (Fig.52). Vacuolation appears in the pith between the second and third plastochrones and is quite evident at the insertion of leaf 2. After the fourth plastochrone the leaf traces can be seen (as procambium) running horizontally in the cortex. The full stelar pattern is seen after the insertion of leaf 4. At this stage there are some large empty cells with some dark staining material in them on the pith side of the vascular segments: later they are seen on the cortical side also. The first protoxylem elements are visible (in the trace of leaf 5) after the tenth plastochrone, distance from apex about 450μ. The first periclines appear opposite the files of xylem in the same trace after I4 plastochrones, distance 700μ. Lower down in the same internode (I000μ) periclines also begin across the files of parenchyma. The active state of growth of the material was shown by the presence of numerous nuclear divisions in all tissues. (See Figs. 50,5I,52,58,59).

Type 5. SALIX. Stelar pattern CRX'0.

The series taken was from a shoot apex in active growth so that bud-scales were not included. The spiral phyllotaxis is shown in Fig. 53.

Leaves I and 2 join the axis at 10μ and 20μ from the apex respectively: leaf 3 joins at 70μ and leaf 4 at 80μ: internodal extension begins between leaf 4 and leaf 5 which joins at 180μ: leaf 6 joins at 370μ.

Vacuolation of the pith is seen after two plastochrones, distance from the apex 40μ. The stele is marked out as procambium after four plastochrones, distance 80μ. (See Fig. 55).

The first protoxylem elements are seen after five plastochrones in the median trace of leaf 4 while in the median trace of leaf 5 at the same level there are several small touching xylem elements. (Fig. 56). Radial division in the file of parenchyma adjacent to the xylem are seen in the same figure. Periclinal divisions opposite the xylem are seen in the same trace lower down in the same internode (Fig. 57) and the cambium is completed across the segment in the median trace of leaf 5 at about the same level (270μ from apex). The first bud-meristem is opposite leaf 5.

At 470μ from the apex several rows of secondary xylem are seen in the median trace of leaf 6, also protophloem and dark staining tannin-filled cells. The series was continued for a further 100μ but no interfascicular cambium was seen.

Type 6. BETULA. Stelar pattern CRX'O.

In the fully developed twig of Betula the leaves are spirally arranged, but in the bud the lower (outer) but scales and leaves afe distichous, passing over to spiral in the youngest leaves and stipules. The bud scales here are stipules: a complete resting bud includes 4-6 outer bud scales and about 6 formed leaves, in addition to stipules and the bud apex. Fig. 54. shows the members included in the series studied.

The following is the order of junction of parts:-

L,leaf: i, inner stipule: o, outer stipule.

Joining member:	Plastochrones:	Distance from apex: μ.
apex -	-	-
LI + iL	I	40
oI	2	50
i2	3	80
L2 + o2	4	90
i3 + L3 + o3 + bud 3	5	200
i4 + L4 + o4 + bud 4	6	360
L5 + bud 5	7	II40
L6 + bud 6	8	2340

Vacuolation of the pith is seen after I plastochrone and the stele is procambial after 4 plastochrones (80μ) .Further down in the same internode (I90μ from apex) the first xylem elements are seen in the median trace of leaf 2. Periclines appear opposite the xylem after 6 plastochrones, distance fromapex 360μ. Periclines appear in the parenchyma after 8 plastochrones. Lower down in the same internode cambium is complete in the larger segments though not in the smaller. (See Figs. 54, 60).

Type 7. ACER PSEUDOPLATANUS. Stelar pattern DRO.

Acer is a tree type. The phyllotaxis is opposite and decussate. The bud-scales are leaf-bases and arranged in the same fashion as the foliage leaves. The series studied is from a lateral bud: it will be seen that the members on the adaxial side of the system join the bud axis in advance of the abaxial members. (Figs. 61,62).

The leaf-gap is trilacunar. The node of Acer has previously been described and figured by the writer.(34).

The stele is more dispersed and the procambium deeper than in Tilia, Salix, Betula and Ulmus, but the xylem differentiation is of the regular type. The secondary stele is open.

The pith is defined just below node I (70μ) and the stele marked out after node 2 (90μ). After node 3 the traces of leafI are still procambial while the first signs of differentiation are visible in the median traces of leaves 2 and 3. (Fig.63). In this case protophloem appears first. After node 4 (230μ) protophloem is well defined, three large empty elements being seen. (Fig.64). Two or three protoxylem elements are also seen. After node 5 (400μ) the xylem is well defined: periclinal divisions opposite the xylem have given 2-3 secondary elements: one radial division has taken place in the parenchyma and the first sign of periclinal division is seen here (Fig.65). After node 7 the vascular cambium is complete: several rows of secondary elements are seen in both the xylem and parenchyma files (Fig.66, distance from apex I200μ). The series was contin ued through node 8 and the internode below but even at 4000μ from the apex no interfascicular cambium was seen.

Examination of an older shoot (June) on which four pairs of expanded leaves had developed enabled the study of differentiation to be carried a stage further.

Interfascicular cambium begins below the second pair of expanded leaves. The internode was about 3 inches long and the

beginning of cambial activity across the gap-residues was seen about the middle of the internode. The cambium ring was complete just above the insertion of the third pair of leaves. About ten xylem elements had been formed in rádial rows, of which only the four oldest were lignified. The outer pericycle showed the first stages of development of sclerenchyma, four to five rows of regular cells which had lost their contents but which had not yet become lignified. These cells were in contact with a definite starch-sheath, which was already tangentially strained. In the internode below the fourth pair of leaves the pericycle was thickened and lignified and the starch-sheath collapsing. At the same level the phloem was well differentiated with large sieve tubes, small companion cells and phloem parenchyma.(Fig. 67,68,69).

Type 8. SAMBUCUS NIGRA. Stelar pattern DIB.

Sambucus nigra is a tree of moderate size. The leaves are large, compound and stipulate: the phyllotaxis is opposite and decussate. The bud-plan is shown in Fig.70.

Five vascular strands enter from each leaf, three main leaf-trace strands and two stipule traces; the latter fuse on entering the stelar ring so that the contribution at each node is four strands(not counting the bud-steles).

In longitudinal section the apex is seen to be massive and blunt: this section (Fig.7I) shows the stem at its greatest radial expansion, with two newly formed soubassements.

The tunica is of two layers. In the third and subsequent layers of the central core active horizontal and vertical divisions are in progress, giving 'quartettes' of pith cells, which enlarge considerably below. About the level of node 3 the pith cells divide by oblique vertical as well as horizontal walls and intercellular spaces begin to appear. The average diameter of the pith cells in the fourth internode is double that at the apex, and as the cells are more or less isodiametric this represents a volume increase of about eight times. The resulting pressure on the procambium can be well seen by comparing cells at the level of the first, second and third nodes. At the level of node I the procambium cells are about 45μ x I0μ and the end walls, though oblique, form definite angles with the long walls. The nuclei are rounded-oval in form. At node 2 the procambium cells are spindle-shaped, about 55μ long, and when in division show crowding and displacement of the chromosomes. Fig. 72a shows the condition at node I; Fig.72 b and c show nuclear divisions just below node 2; Fig.72d shows the condition at node 3. One protoxylem element is to be seen: note the 'vermiform' nucleus in the fourth cell from the protoxylem: this is probably a cambium cell.

The procambial strands are isolated and surrounded by fully vacuolated parenchyma at the level of node 2 (40μ from apex). The strand is about 6 cells deep by 6 cells wide at its maximum, tapering towards the pith (Fig.73). The first protoxylem is seen in the median trace of leaf 3, I00μ from apex. Protophloem appears at I40μ from apex. The next stage to be illustrated is taken from the median trace of leaf 3, distance from apex I70μ (F ig. 74). This shows the scattered differentiation of primary xylem, four elements, not in radial series, being evident. Periclinal divisions have appeared across the whole segment practically simultaneously: possibly the large vessel marked X might be considered secondary.

The massive character of the bud made it necessary to use only very short pieces of material, so that the origin of interfascicular cambium was not obtained in this series.

Further information was gained from a shoot still in active extension growth (June). The whole shoot was about I8" long and carried five pairs of expanded leaves in addition to those folded over the apex. Considerable extension both in length and in diameter was found in passing from the apex to the base of the shoot. The first internode below the apical bud was about 2mm.long and Imm. in diameter: the section was hexagonal. The first pair of leaves below the apical bud was just beginning to unfold and the petioles were still short. The second internode was about I0mm. long, I.5mm. in diameter and hexagonal. In the third internode great increase both in length and diameter was seen. The length of the internode was 40mm. and the diameter increased from 3mm. at the top to 7mm at the base. In this third internode the stele was well defined as discrete vascular segments separated by gap-residue parenchyma. This amounted to 35% of the stelar ring, so that this ranks as a dispersed stele. Interfascicular cambium

appeared about half-way down the internode and one or two secondary elements had been formed both in the original bundles and in the joining segments at the base of the internode. In this same internode also a line of dark-coloured strained cells was seen near the outside of the pith. This is evidently a sign of breakdown, because lower down in the shoot the pith ruptures about this region and the centre breaks away on sectioning (Fig.75).

In the fourth internode the stem had increased in diameter to 7.5mm. and had rounded out, losing the hexagonal outline of the younger internodes. The joining segments of the stele were well developed, being about 8 cells deep and files of parenchyma (secondary 'wood-rays') penetrated about 2-3 cells in from the cambium. The tangential strain caused by the expansion of the pith and the large lign ified elements of the secondary xylem is well seen at the meeting of the pith, original bundle and joining segment shown in Fig.76.

After this first sudden expansion of the stem the lower internodes increase more slowly: the fifth internode was 9mm.and the sixth IImm. in diameter.

In regard to other secondary developments, it was found that the phloem was not large in amount and contained a high proportion of parenchyma while the sieve tubes were small.

A starch sheath was distinct in the third internode, becoming much distorted towards the base of the shoot. In contact with this sheath were groups of very large empty cells in the outer pericycle. These cells were first visible in the third internode: they did not lignify even at the base of the shoot, but became collapsed and distorted (Fig.77).

It is interesting to note that chlorophyll was found in the cells of the pith border surrounding the original leaf-trace segments, persisting to the base of the shoot. A similar condition was noted by Thoday (38) in Helianthus.

Sambucus makes an interesting contrast to the other members of the Caprifoliaceae in its anatomical structure. It is the largest-growing type (the nearest in size is Viburnum) and it has the most dispersed stele. The others have very beautiful regular, close-set vascular segments, smaller piths and more highly developed pericycles. One is tempted to describe Sambucus as a 'herbaceous tree.' Size in the primary shoot has been gained at the expense of rigidity, by increasing the proportion of parenchyma to vascular and mechanical tissue. Moreover extension growth is achieved very rapidly. As a result the shoot at this stage depends upon turgescence to maintain its rigidity, as can be seen by its rapid wilting; a shoot similar to the one described had wilted between the time of picking and bringing into the laboratory, a matter of minutes. Then the rapid extensionsion of the interfascicular cambium across the gap-residues and the formation of joining segments between the original bundles is quite comparable to the secondary growth of a woody herbaceous stem. A possible correlation might be suggested between the high proportion of parenchyma in the stem and a large auxin supply from the large compound leaves, which quite overshadow the axial part inthe apical region. (In other Caprifoliaceae the leaves are relatively smaller). Sambucus is considered more primitive than the other members of the Family in its floral construction : its anatomy is also divergent.

Type 9. VINCA MINOR. Stelar pattern MRO.

Vinca minor is a woody perennial of more or less prostrate habit. The phyllotaxis is opposite and decussate, the leaf-gap unilacunar. Like other members of the Apocynaceae it possess non-articulated latex tubes in pith and pericycle, also medullary phloem.

In order to escape the flower buds it was necessary to fix material very early in the season. Consequently the series showed fewer nuclear divisions than usual and signs of axillary bud-meristems were late in appearing.

Longitudinal sections of a February bud showed five pairs of leaves in the plane of the section but no buds: the procambium appeared quiescent and few nuclear divisions were seen even in the apical meristem. The tunica here consists of three layers. Horizontal divisions had clearly taken place in the pith but elongation of the cells had not proceeded except at the lower end of the section. The cells there were about three times the vertical height of those near the apex, but their transverse diameter had not increased. The stem remains slender throughout its growth showing no great increase in girth although some secondary growth of vascular tissue does take place.

In transverse sections the pith is seen to be vacuolated after 2 plastochrones (70μ) and the stele is marked out as procambium after 90μ. It appears as two large arcs corresponding to the first pair of leaves, the gaps for leaves 2 being well defined. The first sign of differentiation is seen at this level in the appearance of large cells with collapsed and deep-staining contents on the outer margin of the procambial strand: these are the latex tubes. The procambium itself is fairly deep (6-7 cells) and the cells are small and still irregular in arrangement(Figs. 78,79).

At node 2 (I40μ from the apex) the breaking-up of the procambial arc by radial parenchymatisation can be seen.(Fig.80). The first protoxylem elements are seen in leaf trace 2 at a level of I90μ in the internode below leaf 2 (Fig.8I). Periclines appear at 450μ in the internode below leaf 3 and at the same level the medullary phloem is clearly visible.(Fig. 82). The ordinary phloem appears shortly after the protoxylem. Comparison of Figures 80 and 8I shows that several cells on the pith side of the protoxylem remain small and only slightly vacuolated. These cells subsequently become divided by oblique anticlinal walls to form the medullary phloem.

The degree of multisection in this stele is not extreme (compare Capparis, Figs. 6,28) but it quite clearly arises before vascular differentiation begins. A similar condition is seen in the Oleaceae, where decussate phyllotaxis, unilacunar gaps and large leaf traces are also found. This is illustrated in Fig.83 from the stele of Jasminum.

Type IO. LINARIA PURPUREA. Stelar pattern DRX'B.

Linaria purpurea is a quick-growing herbaceous stem. The leaves are simple, linear-lanceolate and sessile. The phyllotaxis is spiral but often irregular: sometimes the leaves are so crowded as to simulate a whorled condition. The leaf-trace is single and the stele highly dispersed.

In longitudinal section the apex is seen to be squat.(Fig.85). The leaf-primordia follow each other is such rapid succession that (as seen in transverse section) about five primordia may be attach- to the apex within a depth of about I2μ. The tunica appears to consist of a single layer only. Below the immediate apex the axis increases rapidly in girth, mainly by cell-enlargement in the pith, while extension growth is irregular. The leaves incurve over the apex for only a brief period: thry soon grow upward and outward making an angle of about 30° with the stem. No bud-meristem is visible in the longitudinal section, but the transverse series shows a bud opposite leaf 25.

The series studied included more than 60 leaves: 52 of them are seen surrounding the apex in the bud-plan, Fig. 84.

The procambial ring is marked out by fully vacuolated pith and cortex at the level of insertion of leaf IO, distance from apex I20μ. The limits of the vascular segments are clearly de- fined and the first protoxylem elements are differentiating in the trace of leaf 2#, 360μ from the apex. The phloem is still procambial. The pericycle is very clear at this stage: its cells are still only slightly differentiated and it appears to 'bind' the vascular segments together.(Fig.86).

At 520μ from the apex xylem is visible in all vascular seg- ments while protophloem is only beginning to appear in th oldest.

The first periclinal divisions marking the initiation of vascular cambium are seen in the trace of leaf 30, at 660μ from the apex. At 780μ cambial activity is seen in several segments and at 900μ some secondary xylem.(Figs. 87,88,89.) The pericycle retains its densely staining appearance. Interfascicular cambium does not begin to develope until about 60 leaves have been inserted. Fig. 90 shows the first periclinal divisions in the gap-residue parenchyma. Comparison with sections nearer the apex shows that these cells had been quite fully vacuolated: in many cases the nucleus had taken up a position against the wall of the cell which showed the characteristic large central vacuole. The cells which are undergoing periclinal division appear less highly vacuolated. In place of one large central vacuole there are several smaller ones, and the nucleus occupies a central position once more. The cells are also more stainable. It appears that some process of rejuvenation or de-differentiation has gone on and that these cells are once more entering upon a phase of active growth and division. Further studies on this point are in progress.

From a fairly early stage it is possible to make out a continuous row of larger cells in immediate contact with the pericycle. This is an endodermis, and by the time the interfascicular cambium is forming it is well marked with a casparian strip on the radial walls.

The stelar pattern accords with those found in the rest of the Family(see list of genera, p. 35).

An additional example of a bundle from a highly dispersed stele is shown in Plumbago,Fig.9I: the xylem here is scattered.

Type II. VERONICA AMETHYSTINA. Stelar pattern CRC'B.

Veronica amethystina is a shrubby species, with small crowded evergreen leaves. The phyllotaxis is opposite and decussate. Apices were fixed when the plant was in rapid growth. The bud-apex is illustrated in Fig. 92.

This type was chosen to illustrate the precocious initiation of cambium in the procambial strand before any vascular differentiation has begun.

The leaf gap is unilacunar and the trace consists of a large arc which fits its gap very closely.

The trace of leaf I is still procambial at the second node, and regular periclinal divisions can be seen in the cells next the pith. The greatest depth of the procambial arc is 4-5 cells in the centre of the trace: it tapers off to 2-3 cells at the end. This stage is shown in Fig. 93. The first protoxylem elements (derived from cambium and therefor secondary) are seen below the third node (Fig. 94). Two cells of protophloem are also seen: they do not precede the xylem because at this stage the protoxylem is thickened and lignified while the protophloem is not yet completely differentiated. The drawing was made from cells at the end of the vascular segment, as can be seen by the proximity of fully vacuolated gap-residue parenchyma.

The gap-residues are so narrow, usually only one or two cells wide, that interfascicular cambium is quickly established. The stelar ring in the mature stem is very close and regular in texture.

Similar stages of differentiation were seen in Veronica agrestis, V. Chamaedrys, V. Lyallii. The mature steles of many species were also examined and found to be remarkably similar in general organisation of the stele. It seems likely that this type of differentiation is general throughout the genus.

iv). Discussion.

The types described above in detail were chosen to illustrate the differentiation of the stelar patterns described in Part I and also to cover the most important habit-types. They show that the criteria chosen for the analysis of stelar structure are fundamental, being concerned with the basic conditions at the shoot-apex upon which the construction of the mature stele depends. Although only a small number of types are given, all the genera examined followed the same lines in their development according to their stelar type.

It will be seen that no general rule can be formulated for the sequence of tissue-differentiation in the vascular segment. Sometimes xylem differentiation preceded that of phloem and cambium, sometimes phloem appears first and in a few cases cambial divisions begin in the procambium ahead of any vascular differentiation. There is a tendency for the first condition to be associated with the arboreal habit, while soft herbs and lianes often show the second condition. (This may be connected with relatively larger leaf-size and greater photosynthesis). The last condition (precocious cambium) is rare and reasons have already been given for considering it advanced.

There remains a wide field for experiment in the study of conditions at the shoot apex and their relation to stelar development. M.Snow and R. Snow (35,36,37) have opened the attack by their experiments on the removal and displacement of primordia, and R. Snow by his studies on correlative inhibition (38), b ut experiments on the effect of growth-hormones on stelar differentiation deserve attention. It might then be possible to formulate a truly biological explanation of these morphological and anatomical phenomena which at present one must be content with recording.

SUMMARY.

The stelar patterns found in the dicotyledons have been analysed under three headings:-
a). General stelar plan. b). Differentiation. c). Maturation.

A new terminology based on these considerations is proposed:-
Consolidated: vascular segments amount to 80-95% of stelar ring.
Dispersed: vascular segments amount to 30-80% of stelar ring.
Multisect: the leaf-trace is dissected into separate strands before vascular differentiation begins in procambial stage.

The systematic distribution of the various stelar patterns has been studied throughout the range of the dicotyledon families. This survey shows that arboreal habit, spiral phyllotaxis and consolidated open steles are associated with more primitive floral organisation, while herbaceous habit, cyclic phyllotaxis and dispersed bound steles are associated with advanced floral organisation.

The ontogeny of the stele has been studied in over 70 genera, with particular reference to the origin of xylem and cambium.

Two modes of xylem differentiation have been recognised, regular and irregular.

No general rule can be formulated for the sequence of development of tissues in the vascular segment, but there is a tendency in arboreal types for xylem to precede cambium and phloem: in soft or quick-growing herbaceous stems for phloem to precede xylem and cambium: in some advanced woody herbs cambium precedes both xylem and phloem.

The supersession of primary by secondary tissues in the stele is considered to be the most significant evolutionary trend in the anatomy of the dicotyledon stem.

This condition is only found in the gamopetalae and mainly in herbaceous forms.

It is therefore concluded on anatomical grounds that the herbaceous habit is derivative in the dicotyledons.

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LIST OF ILLUSTRATIONS.

29). Liriodendron: bud plan.x 75.

30). Liriodendron: procambial strand showing first protoxylem.x I000

3I). Delphinium: bud plan. x 35.

32). L.S.apex. x 35.

33). procambial strand. x I000.

34). first protoxylem. x I000.

35). primary xylem and phloem. x I000.

36). vascular cambium. x I000.

37). Tilia: resting bud,plan I. x 35

38). resting bud,plan 2. x 35.

39). opening bud,plan I. x 35

40). opening bud,plan 2. x 35.

4I). opening bud,plan 3. x 35.

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47). first protoxylem. x I200.

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49). cambium becoming established in parenchyma. x I200.

50). Ulmus: bud plan I. x 75.

5I). bud plan 2. x 75.

52). apical meristem. x I000.

53). Salix: bud plan. x 75.

54). Betula: bud plan. x 75.

55). Salix: procambium. x I000.

56). first protoxylem. x I000.

57). vascular cambium,primary phloem. x I000.

58). Ulmus: primary xylem. x I000.

59). vascular cambium,secondary xylem,primary phloem.x I000.

60). Betula: vascular cambium, secondary xylem. x I000.

6I). Acer: bud plan I. x 75.

62). bud plan 2. x 75.

63). Acer: procambium. x I000.

64). protophloem and protoxylem. x I000.

65). initiation of vascular cambium. x I000.

66). completion of vascular cambium. x I000.

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68). part of bundle and adjacent gap-residue showing vascular and interfascicular cambium, phloem, pericycle, starch-sheath. x 500.

69). lignified pericycle and strained starch sheath from base of shoot. x 500.

70). Sambucus: bud plan. x 75.

7I). L.S. apex. x I50.

72). details of procambium from L.S. x I000.

73). procambium, T.S. x I000.

74). primary xylem, vascular cambium, primary phloem. x I000.

75). older stem, plans of internodes 3,5,6. x 75.

76). edge of original bundle and joining segment, showing tangential strain on pith. x 500.

77). pericycle and collapsing starch sheath. x 500.

78). Vinca: bud plan. x 75.

79). procambium, latex tubes. x I000.

80). "Multisection" - division of procambial arc by radial parenchymatisation before differentiation. x I000.

8I). first protoxylem. x I000.

82). inner portion of segment, showing primary and secondary xylem, vascular cambium, medullary phloem. x I000.

83). Jasminum: portion of stele. x I000.

84). Linaria: bud plan. x 75.

85). L.S. apex. x 75.

86). procambium with first protoxylem. x I000.

87). primary and secondary xylem and primary phloem. x I000.

88). vascular cambium, phloem, pericycle, endodermis. x I000.

89). fully organised bundle, pericycle, endodermis. x I000.

90). edge of a bundle showing inception of interfascicular cambium in gap-residue parenchyma. x I000.

9I). Plumbago: fully organised bundle flanked by radially strained parenchyma of gap-residue: note irregular primary xylem, small amount of secondary xylem, vascular cambium, phloem. x I000.

92). Veronica: apical meristem. x I000

93). procambium showing periclinal divisions in cells next pith before vascular differentiation has begun (precocious cambium). x I000.

94). later stage, showing secondary protoxylem, primary protophloem. x I000.

Figure I. T.S. Stem of
Magnolia.
x 35.

CIO.

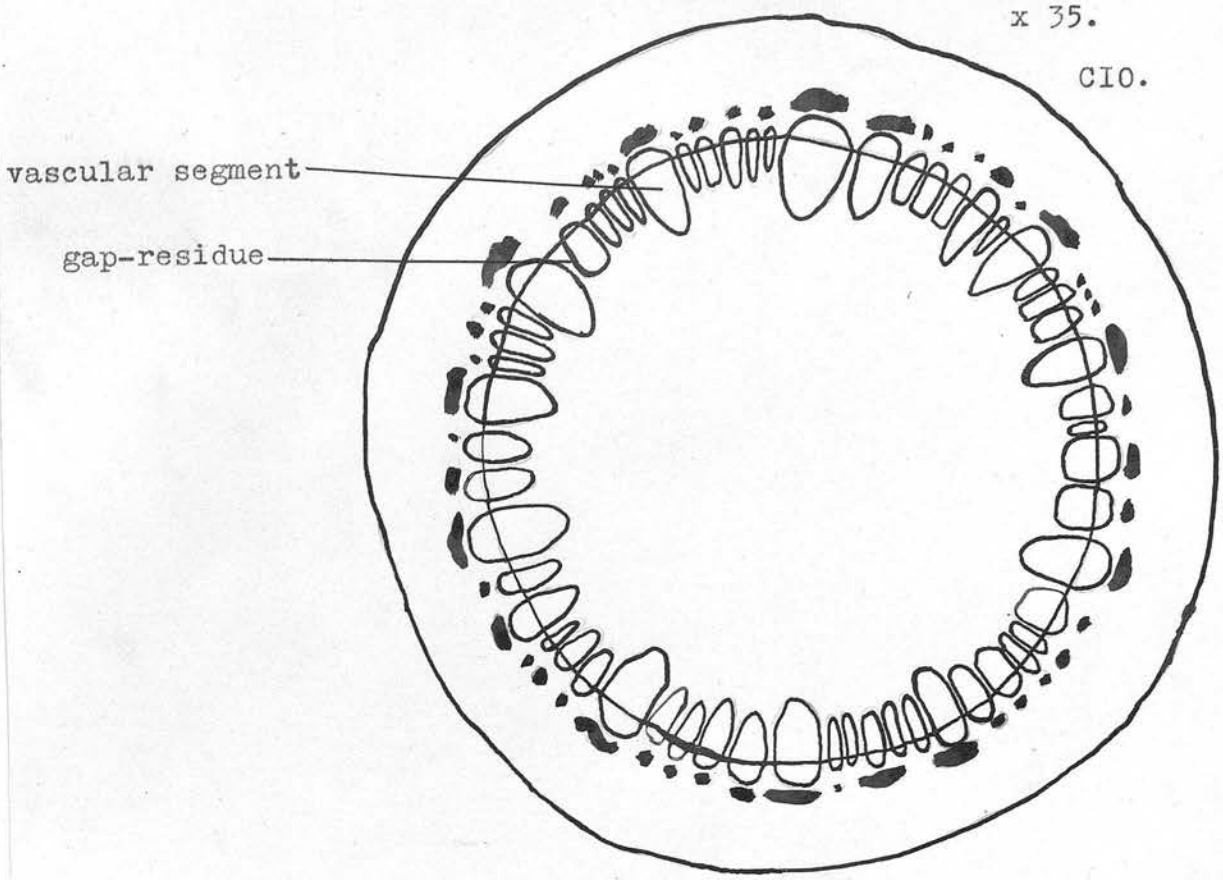


Figure 2. T.S.Stem of Magnolia.x 500

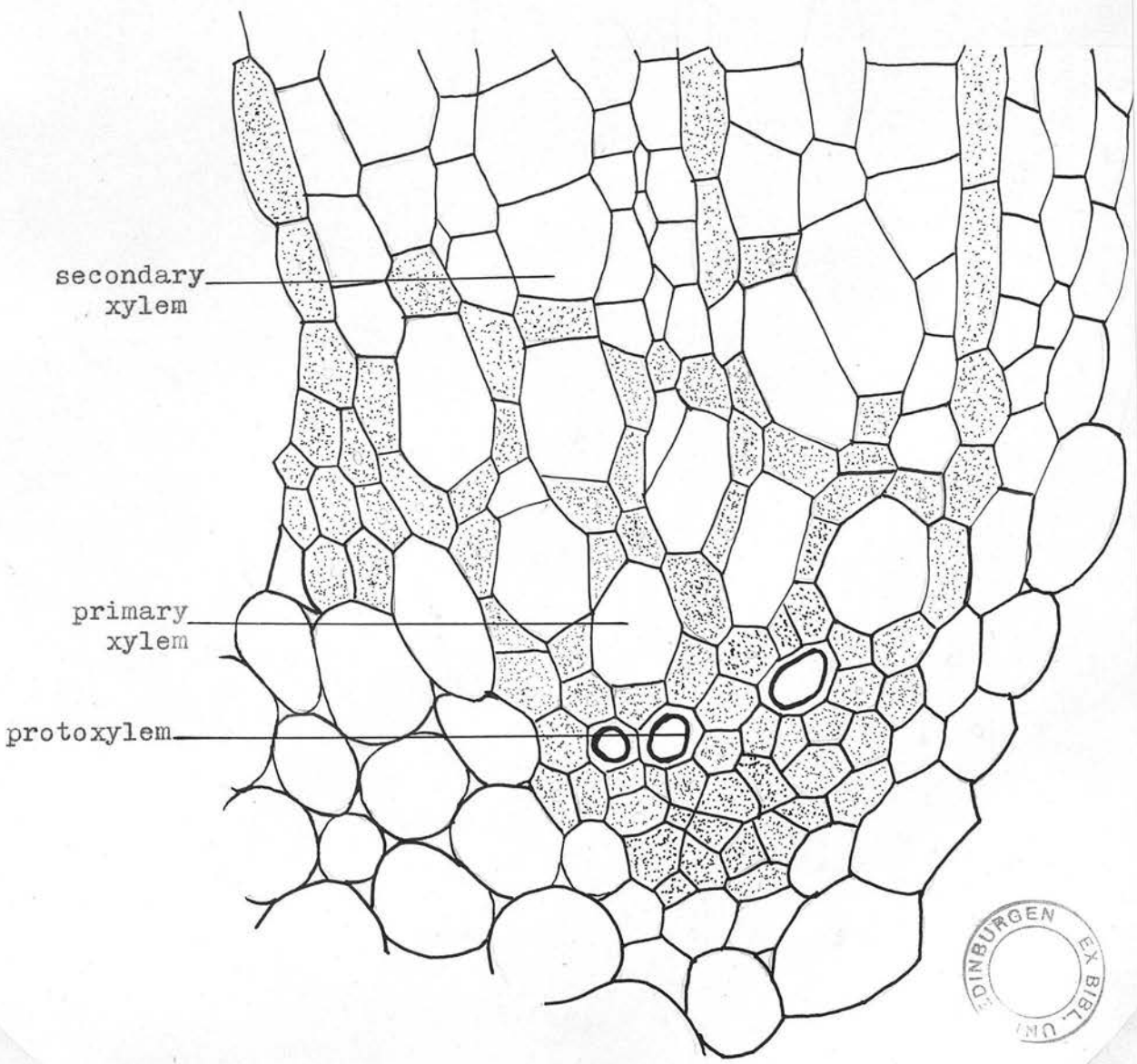
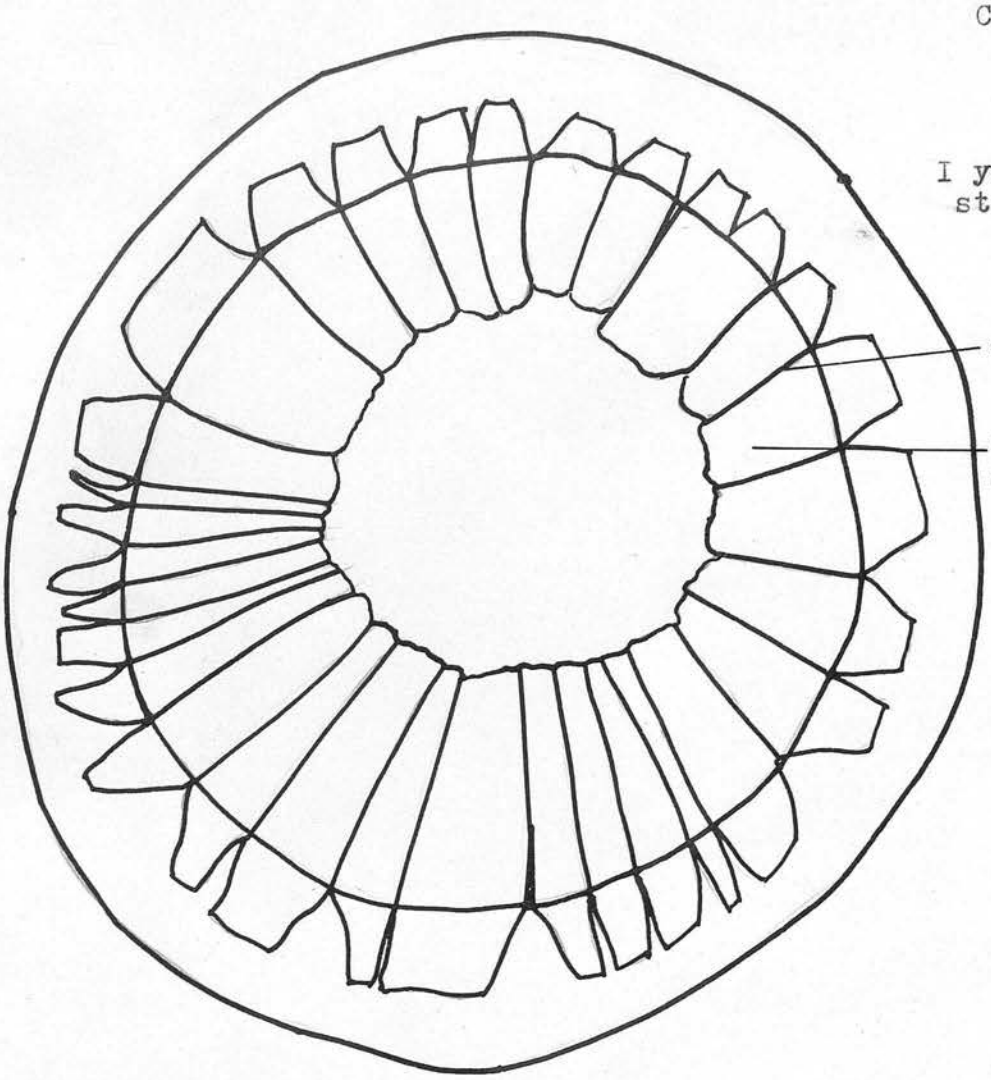


Figure 3. T.S.S. Stem of Tilia.

CRX'O.



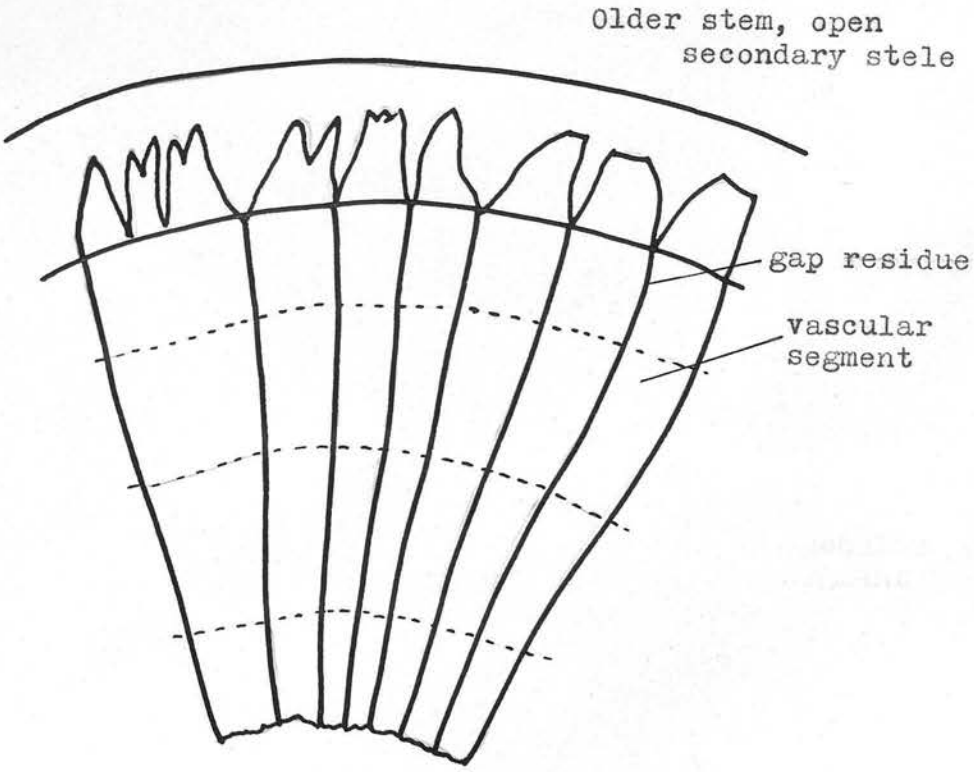


Figure 4.

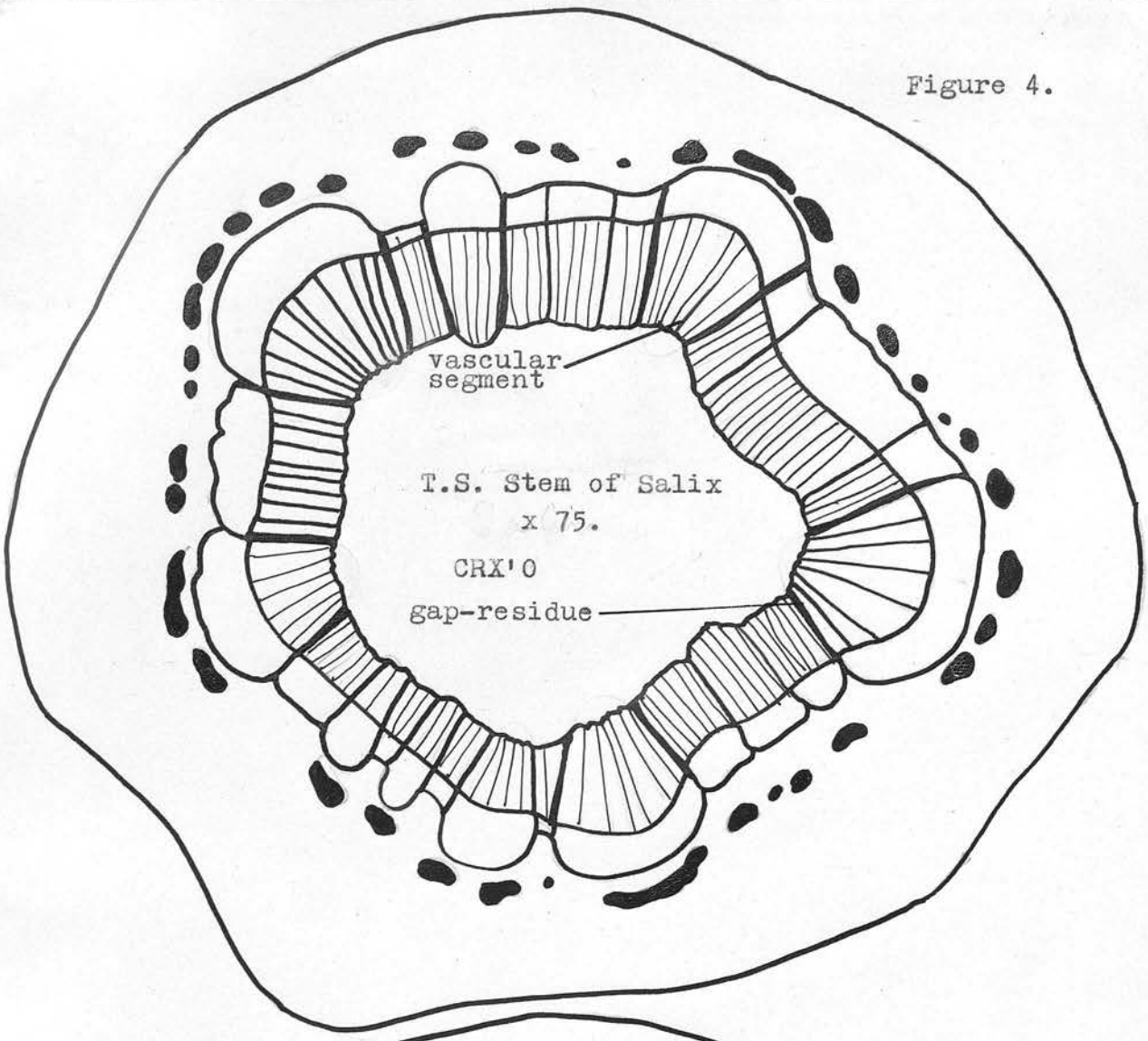
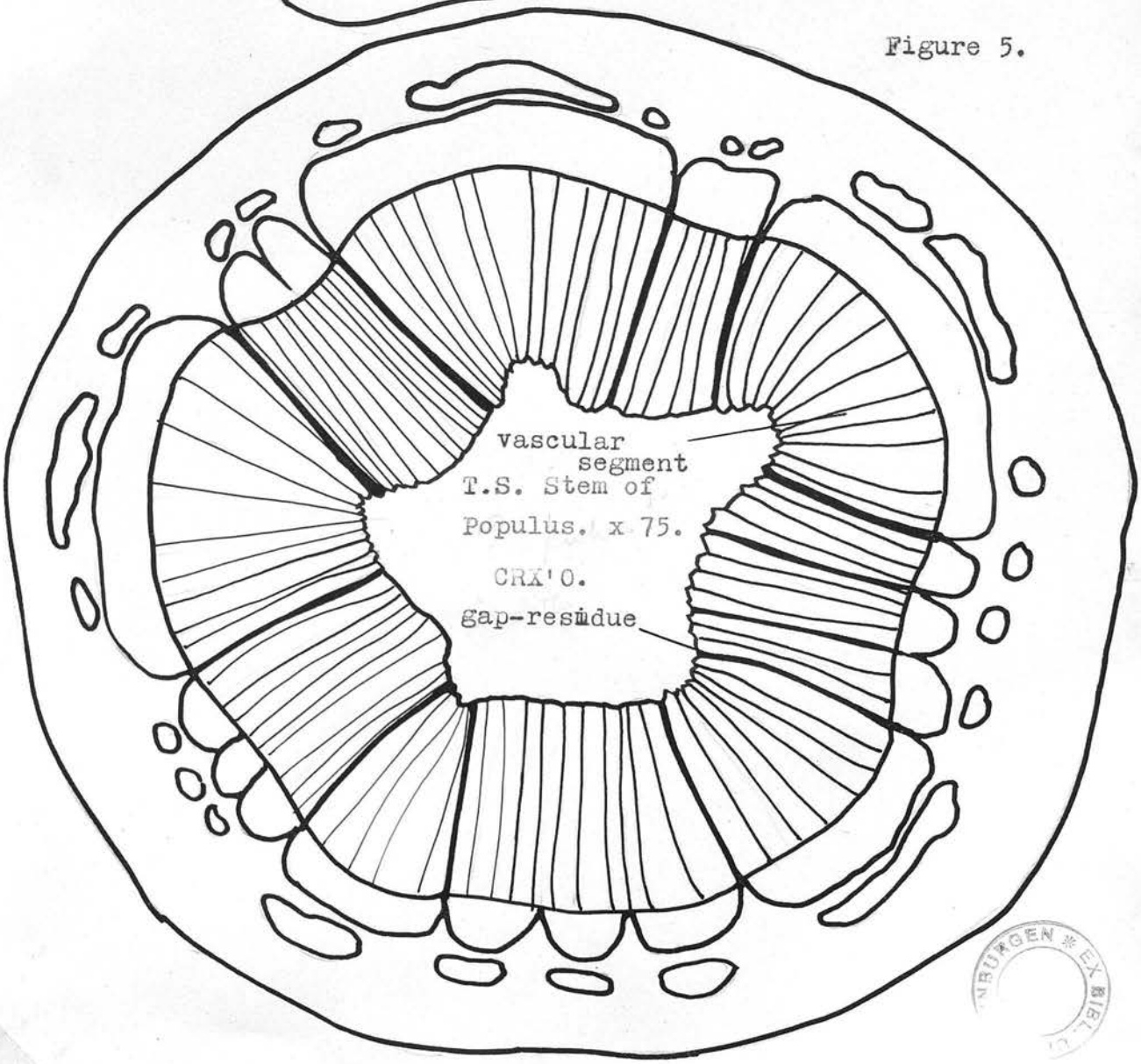


Figure 5.



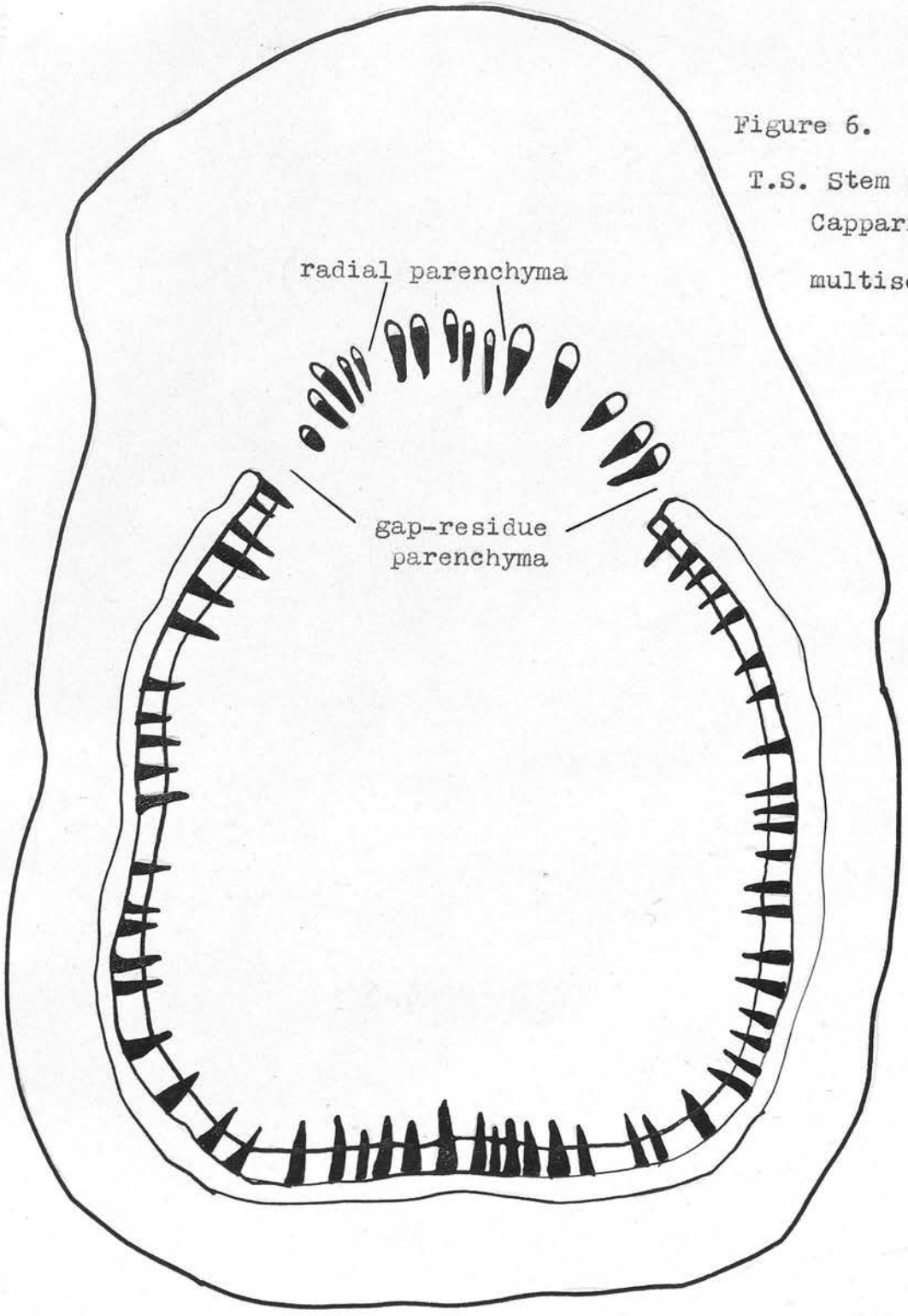


Figure 6.

T.S. Stem of Capparis. x 75.

multisect stele

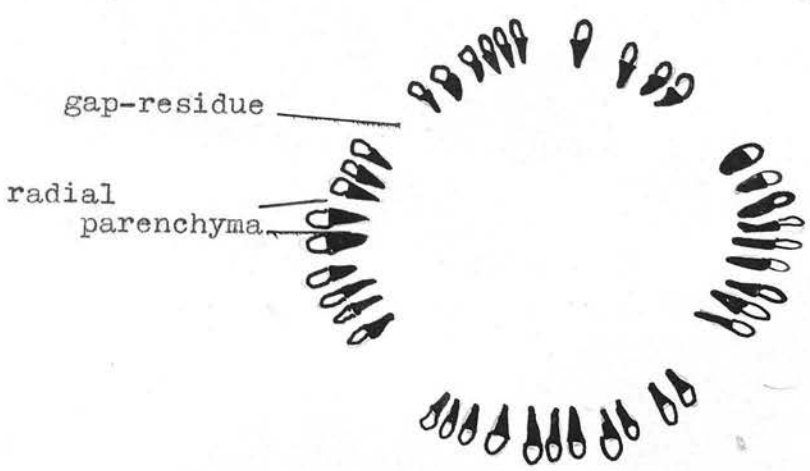


Figure 7.

Multisect stele of Clusia. x 75.

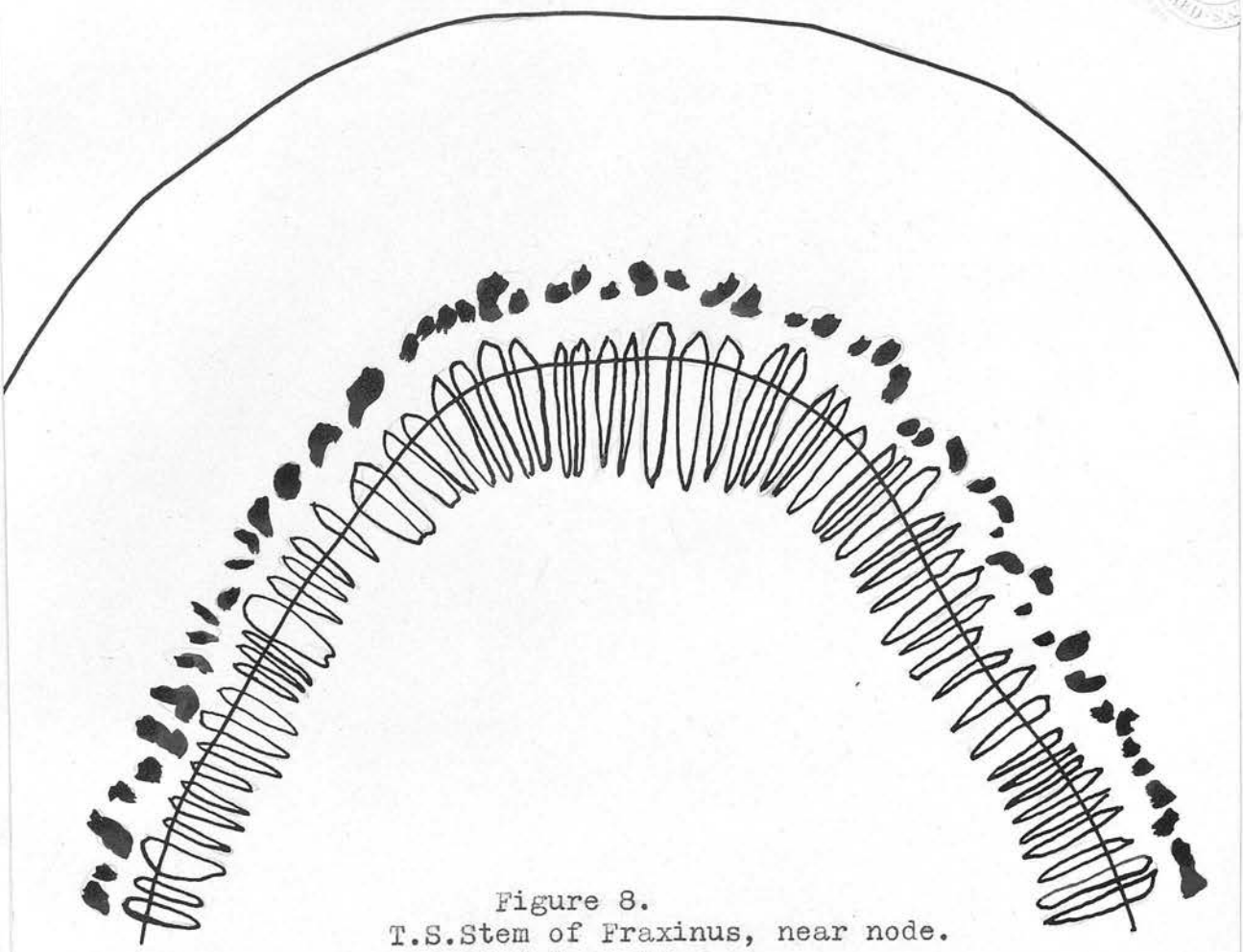


Figure 8.
T.S.Stem of Fraxinus, near node.
M.R.O. x 75.

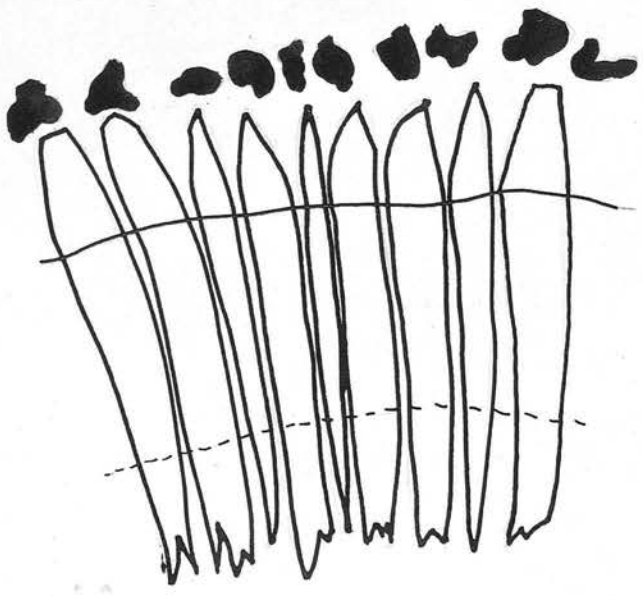


Figure 9.

Older stem of Fraxinus. x 75.

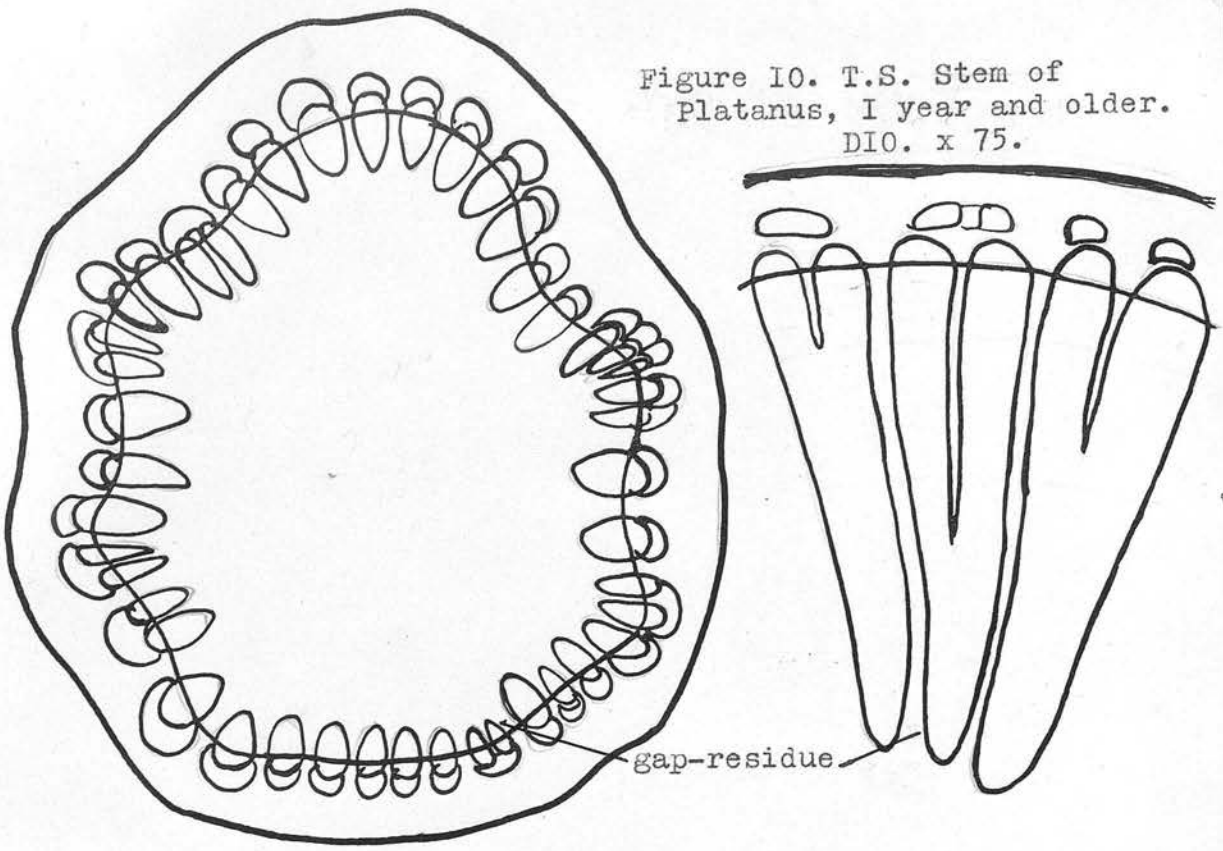


Figure IO. T.S. Stem of Platanus, I year and older. DIO. x 75.

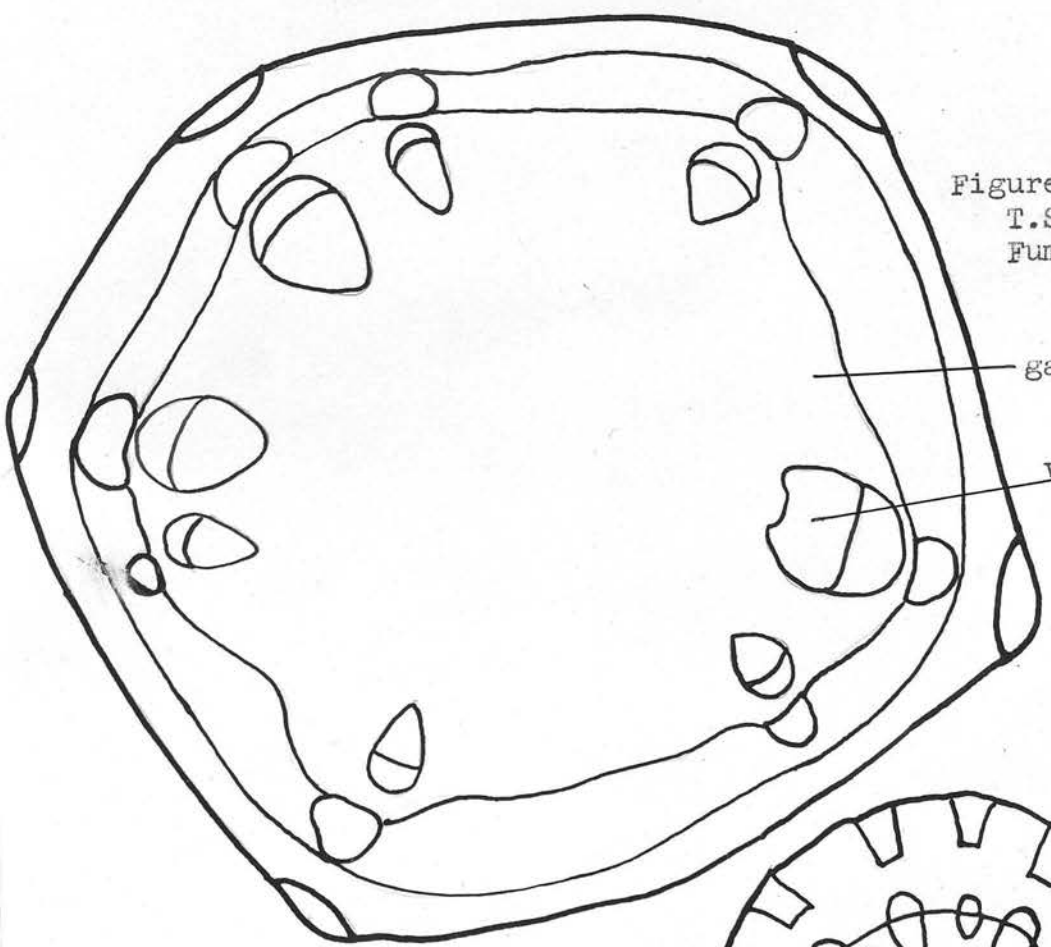


Figure I2. T.S. Stem of Fumaria. x 75. DIO.

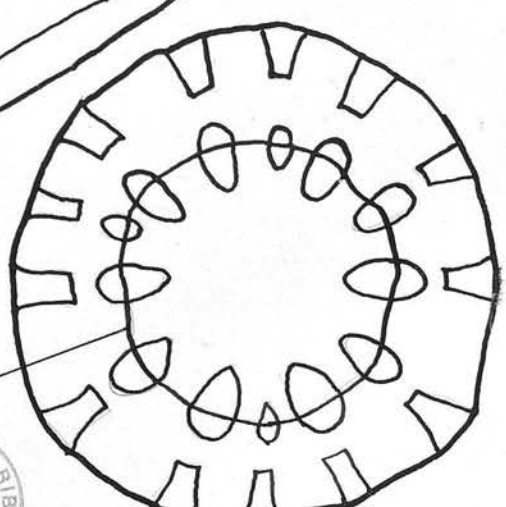


Figure II. T.S. Stem of Clematis. x 35. DIO.

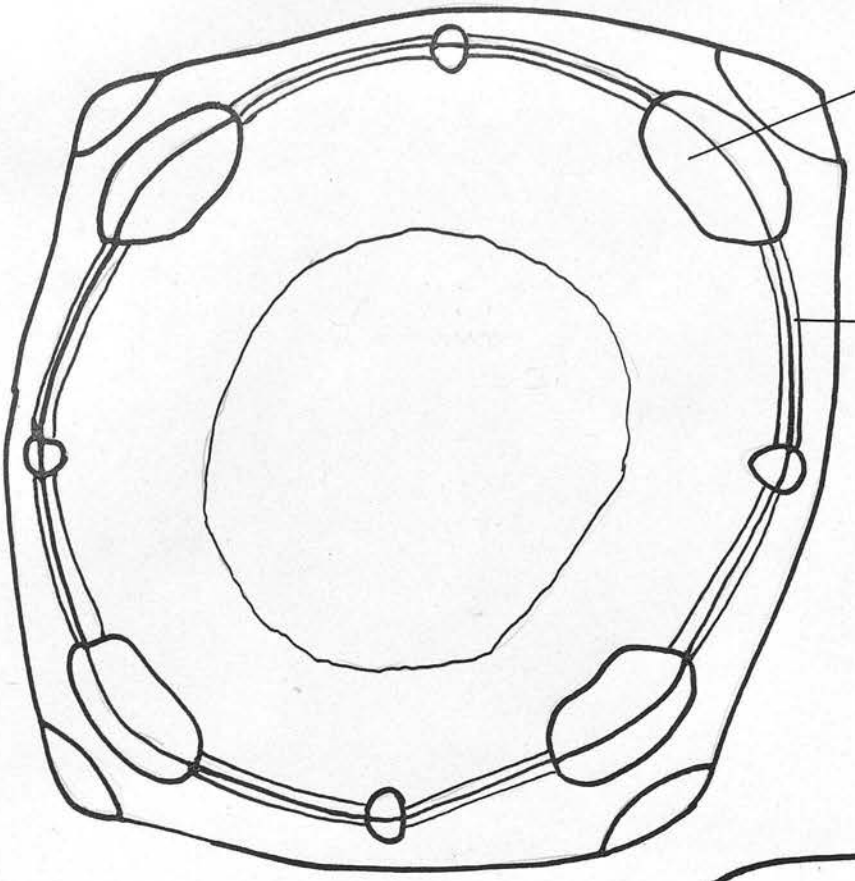


Figure I3.
T.S. Stem of
Lamium. x 35.
DRB.

Figure I5.
T.S. Stem of
Physalis. x 35
DRB.

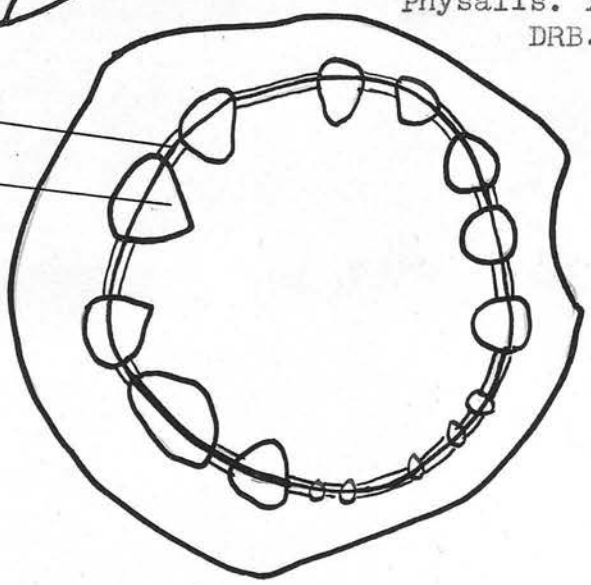


Figure I6. T.S. Stem of
Lonicera. x 35
DRB.

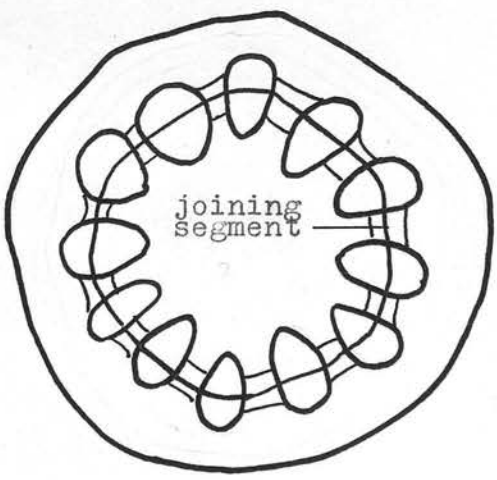
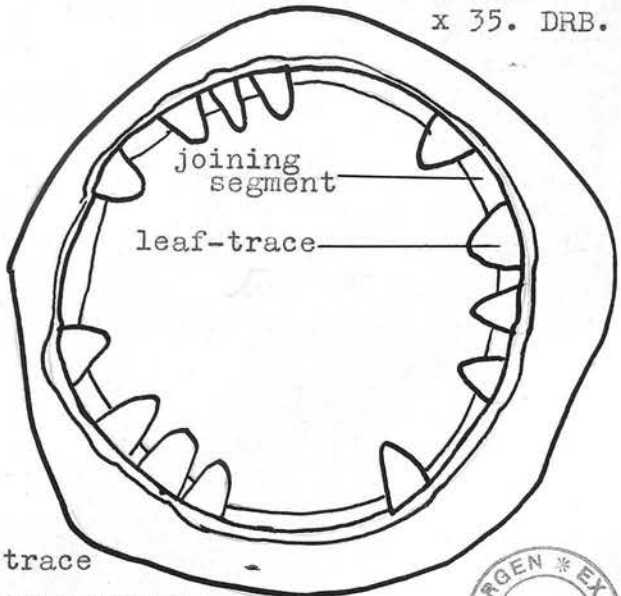
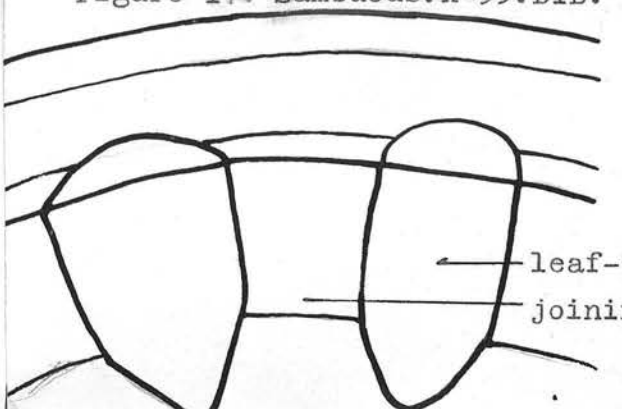


Figure I4. T.S. Stem
of Solanum.
x 35. DRB.



T.S. Stem of
Figure I7. Sambucus. x 35. DIB.



IRREGULAR DIFFERENTIATION OF XYLEM. x 500.

Figure I8.
Delphinium.

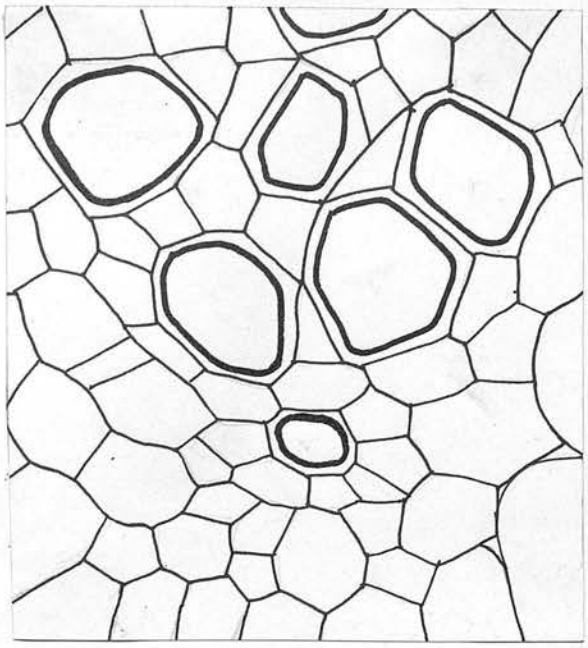


Figure I9.
Bougainvillea.

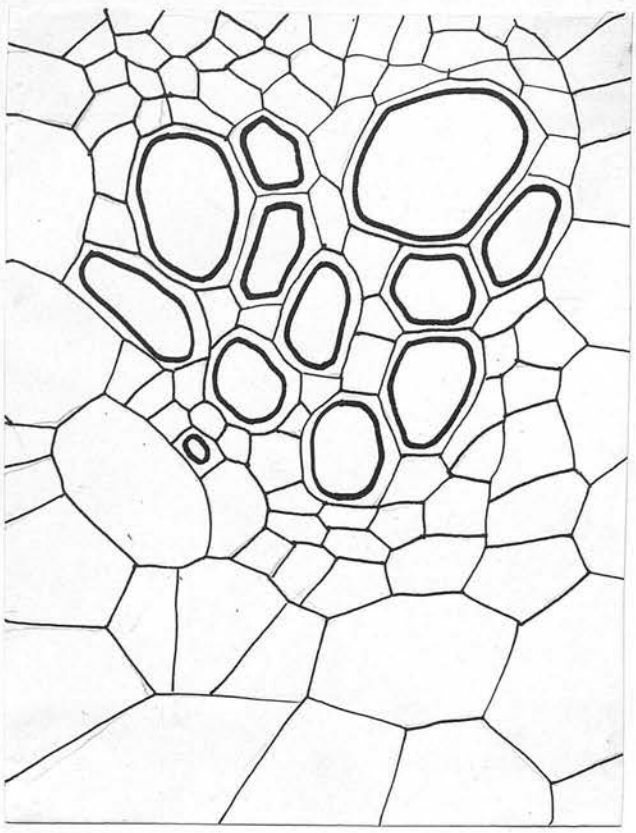


Figure 20.
Dillenia.

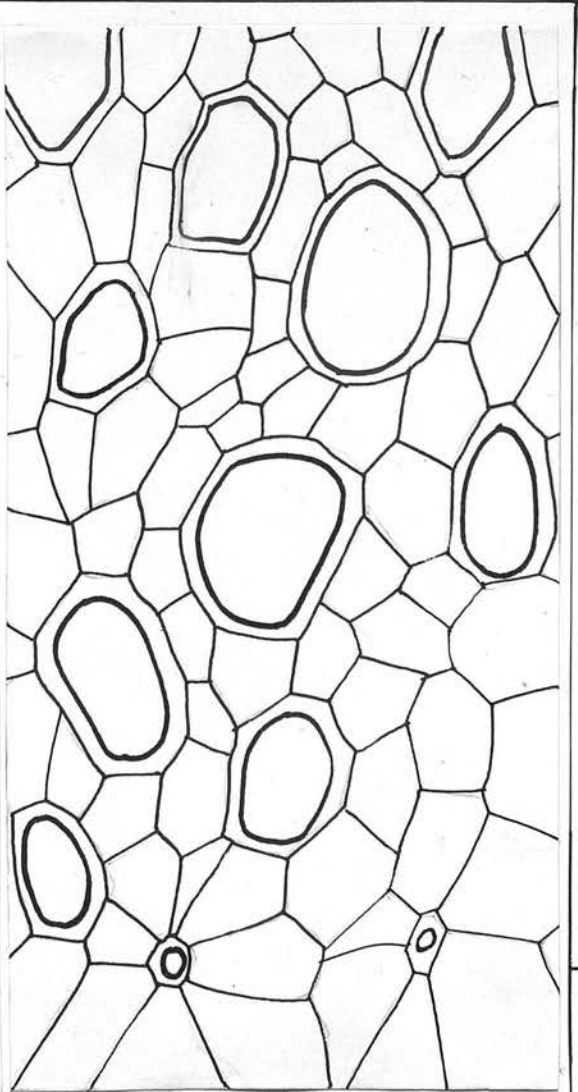
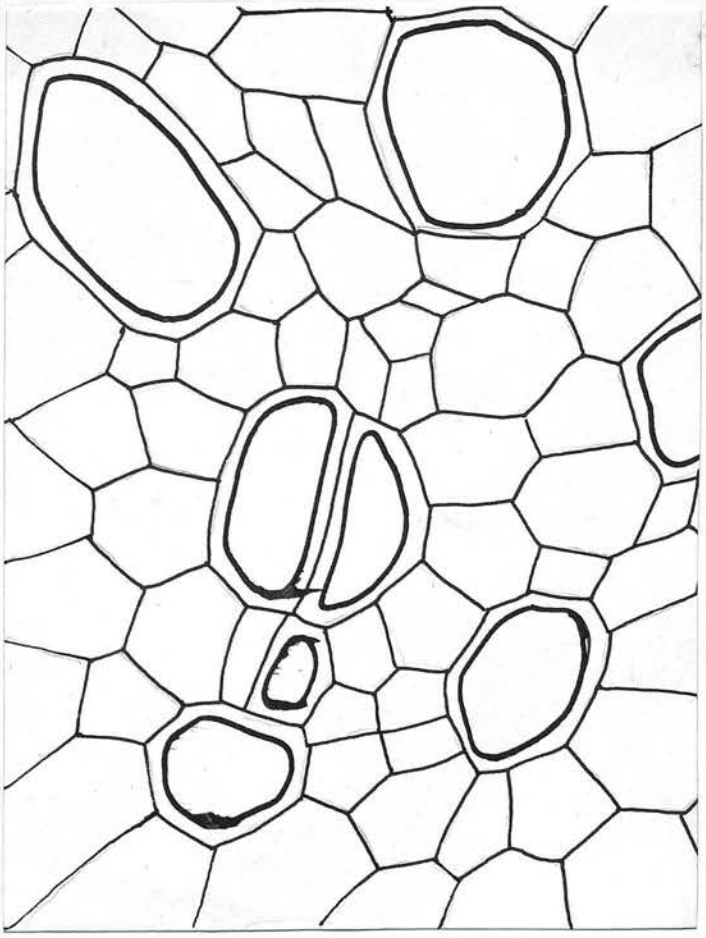


Figure 2I.
Sambucus.



REGULAR DIFFERENTIATION OF XYLEM. all figures x 500.

Figure 22. Ulmus.

Figure 23. Tilia.

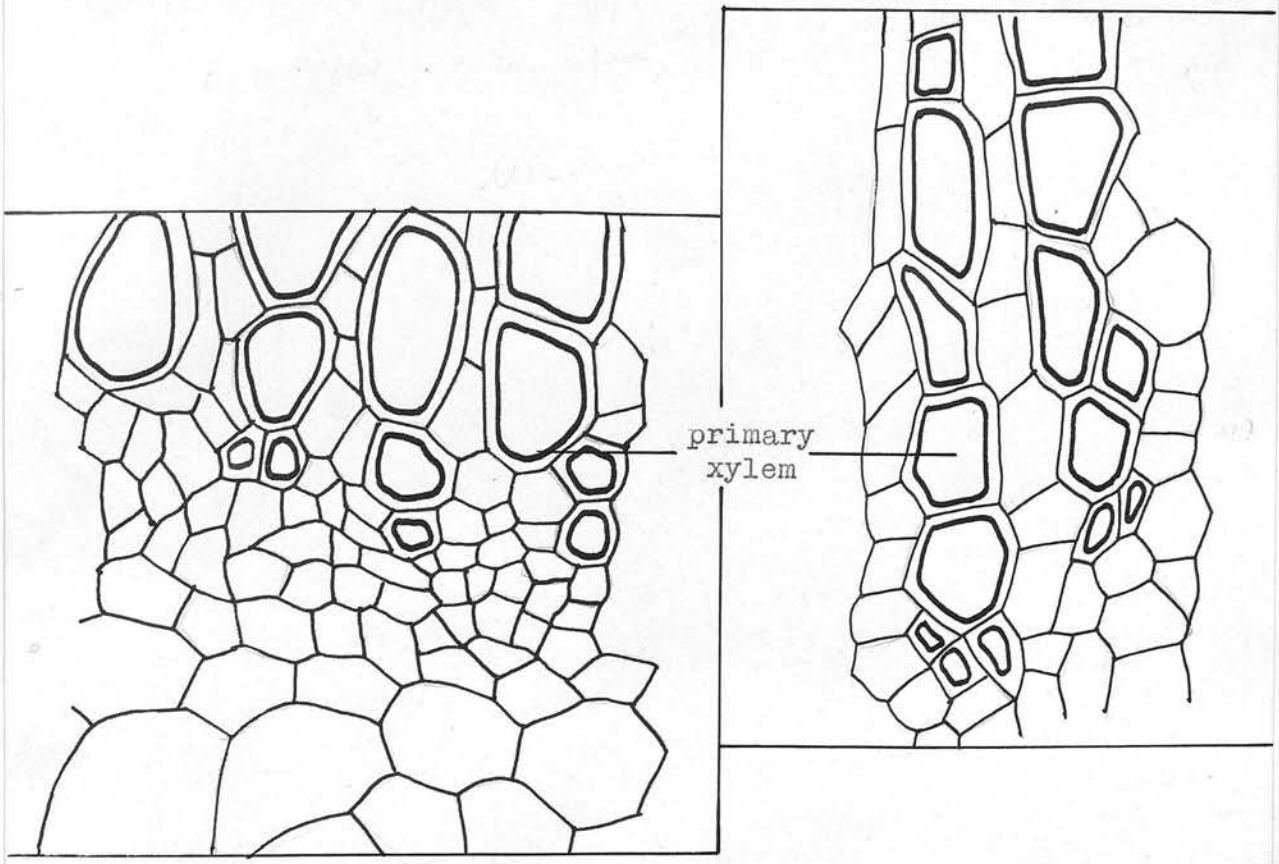


Figure 24. Ceropegia.

Figure 25. Helianthus.

Regular xylem from precocious cambium: all xylem secondary.

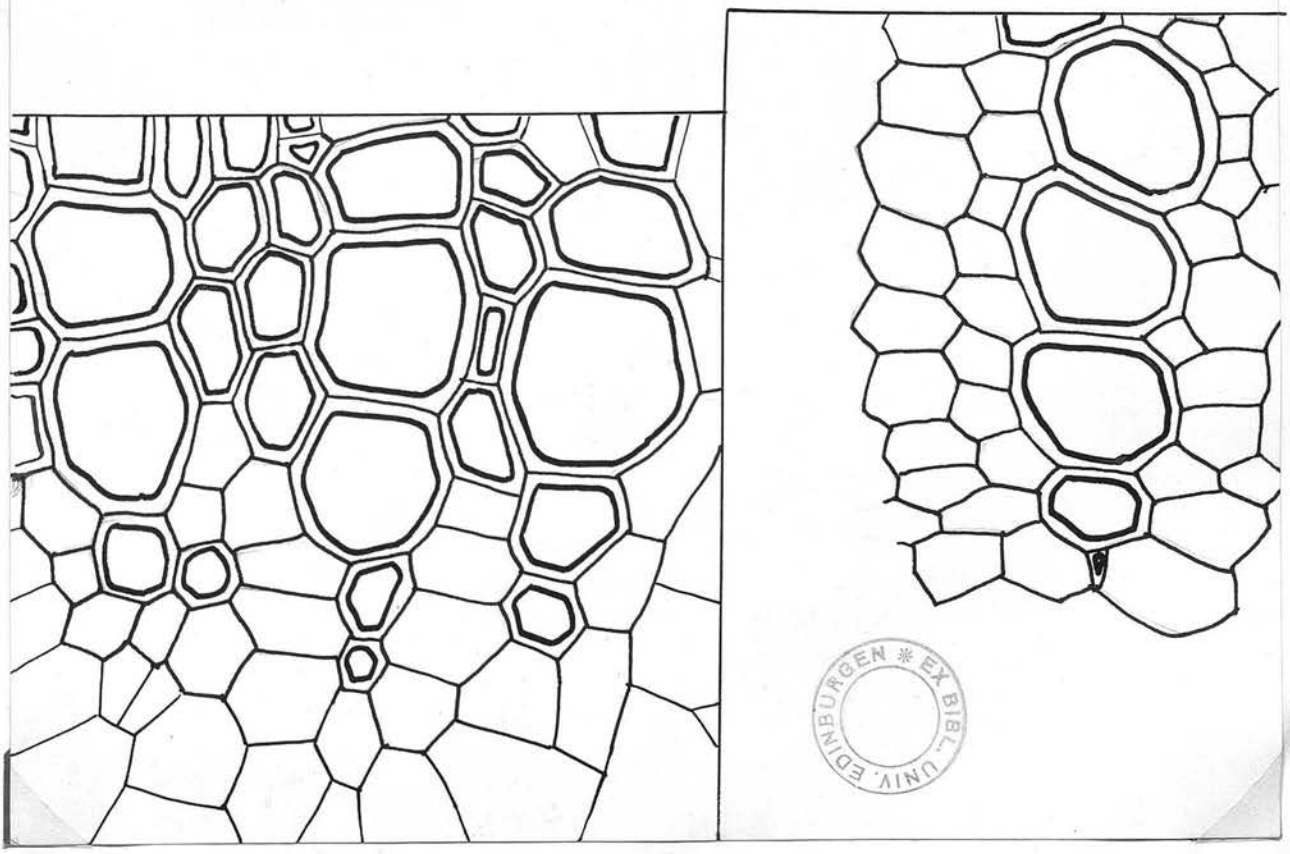
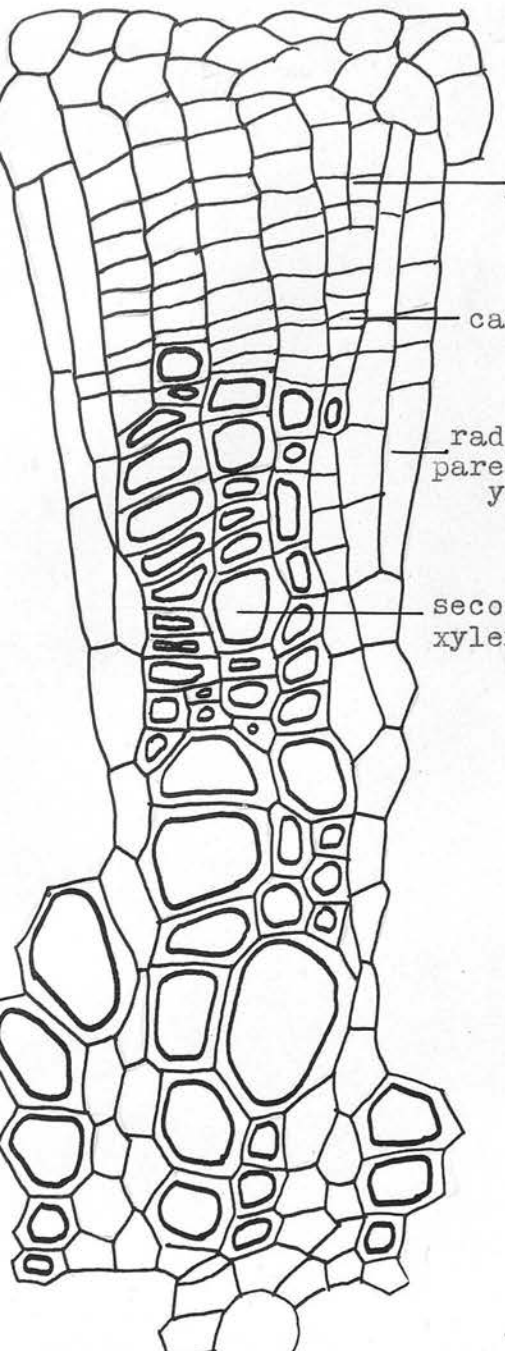
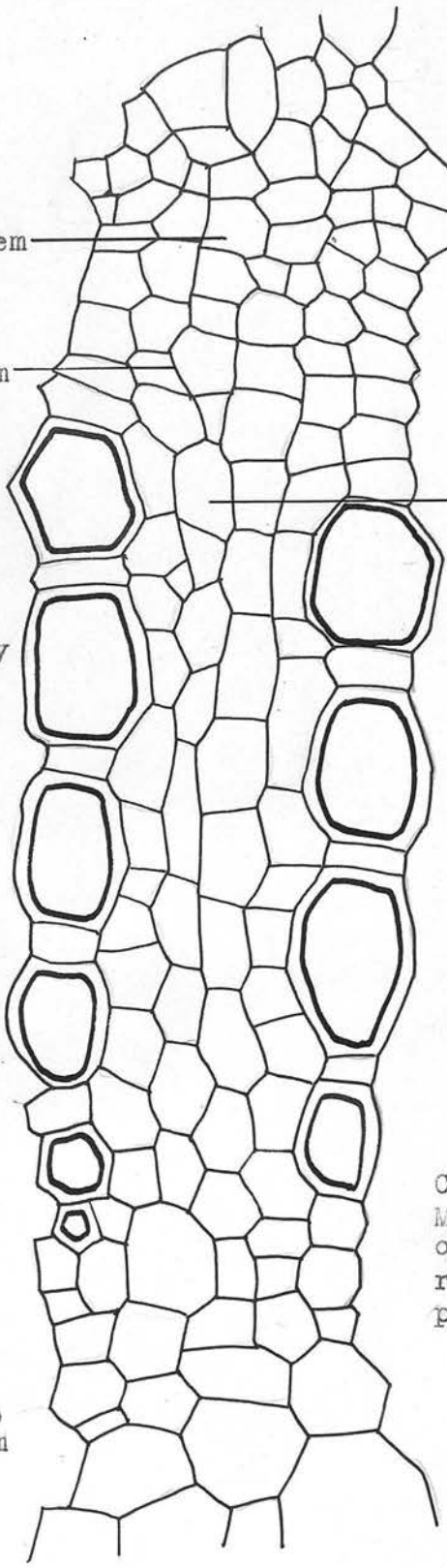


Figure 26.



Fraxinus. Multisect open stele, regular primary xylem

Figure 27.



Clusia. Multisect open stele, regular primary xylem

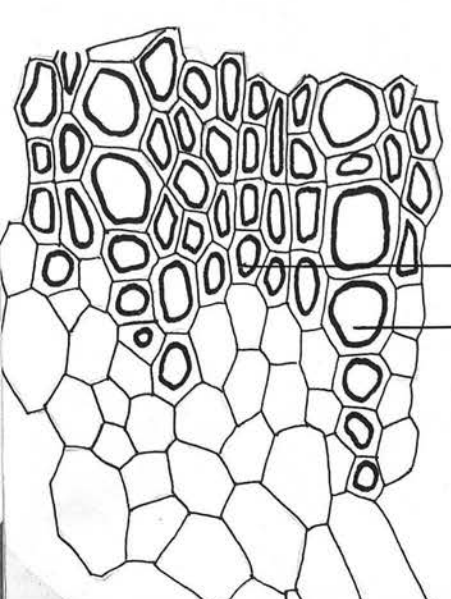


Figure 28.

Capparis. Multisect bound stele, regular primary xylem.

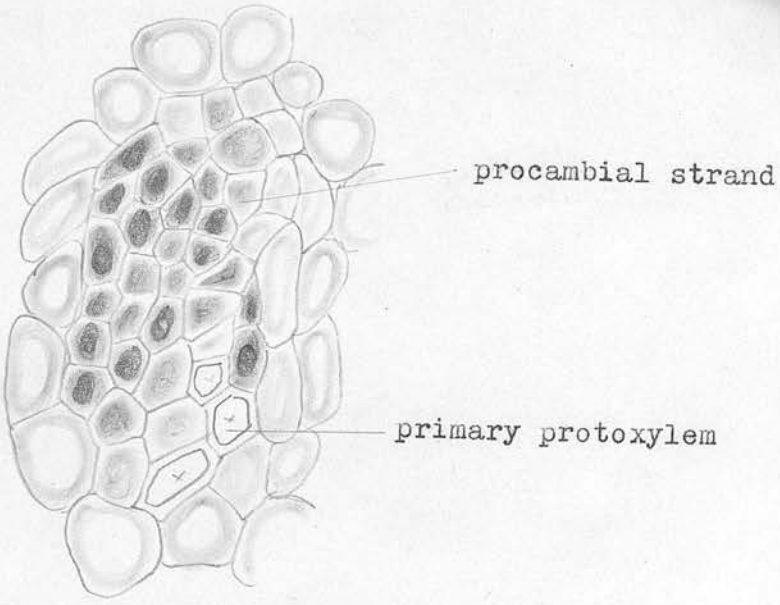
All figures x 500.

In Figure 29 and succeeding illustrations the distance of the section from the apex (where relevant) is marked in μ on each drawing.


Figure 29. LIRIODENDRON. Bud plan. x 75.
1
p2
s2
s2
l1
s1
p1
s1
l2
p3
s3
s3
2
p2
s2
a
s2
p1
3
s2
p2
s1
s2
80μ
4
p2
s3
s3
s2
260μ
p3
5
p2
340μ
p3
p,petiole: l,lamina: s, stipule: a,stem.

Figure 30. (440μ)

LIRIODENDRON. x 1000.



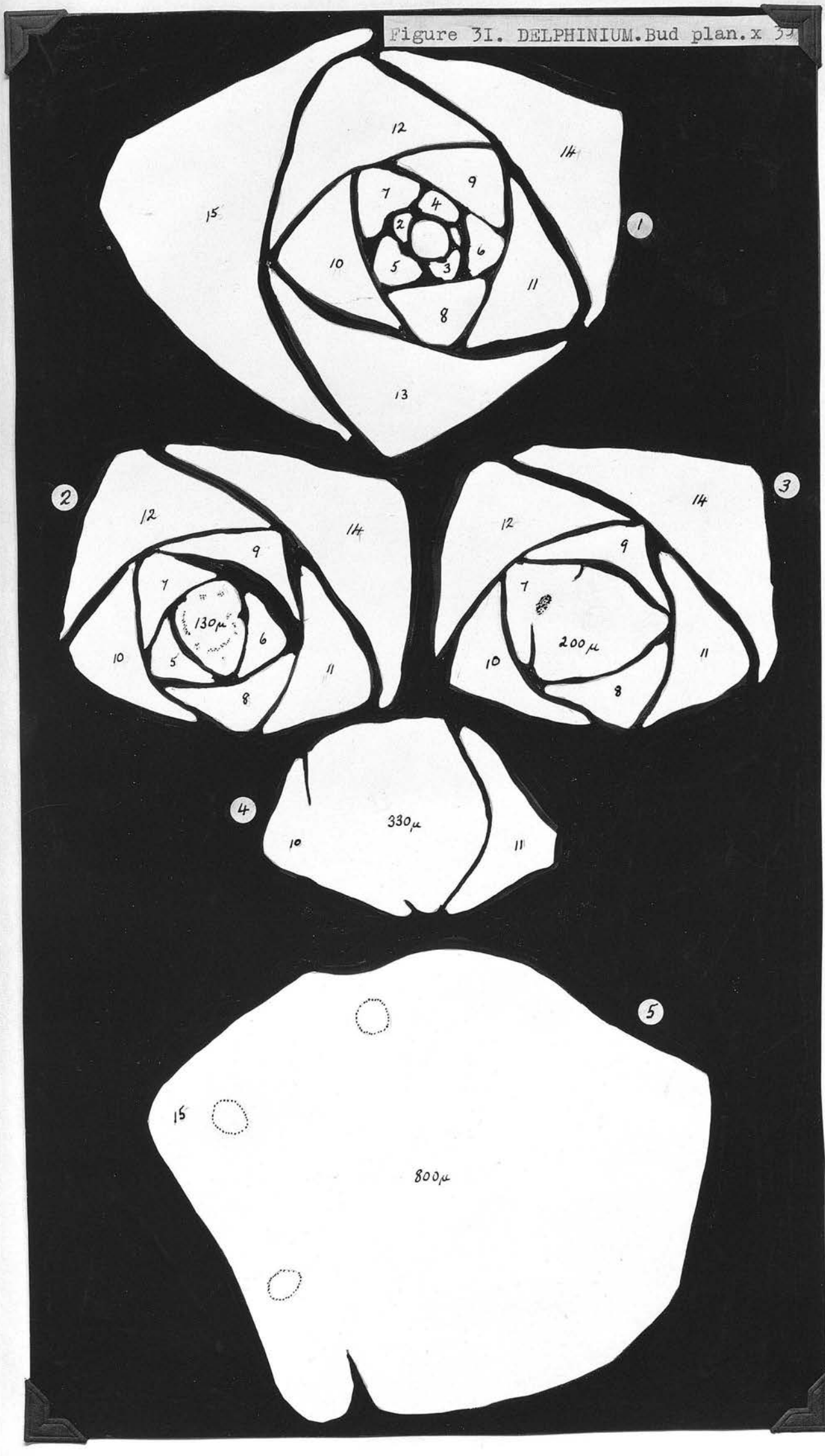


Figure 31. DELPHINIUM. Bud plan. x 3
1
2
3
4
5
130μ
200μ
330μ
800μ

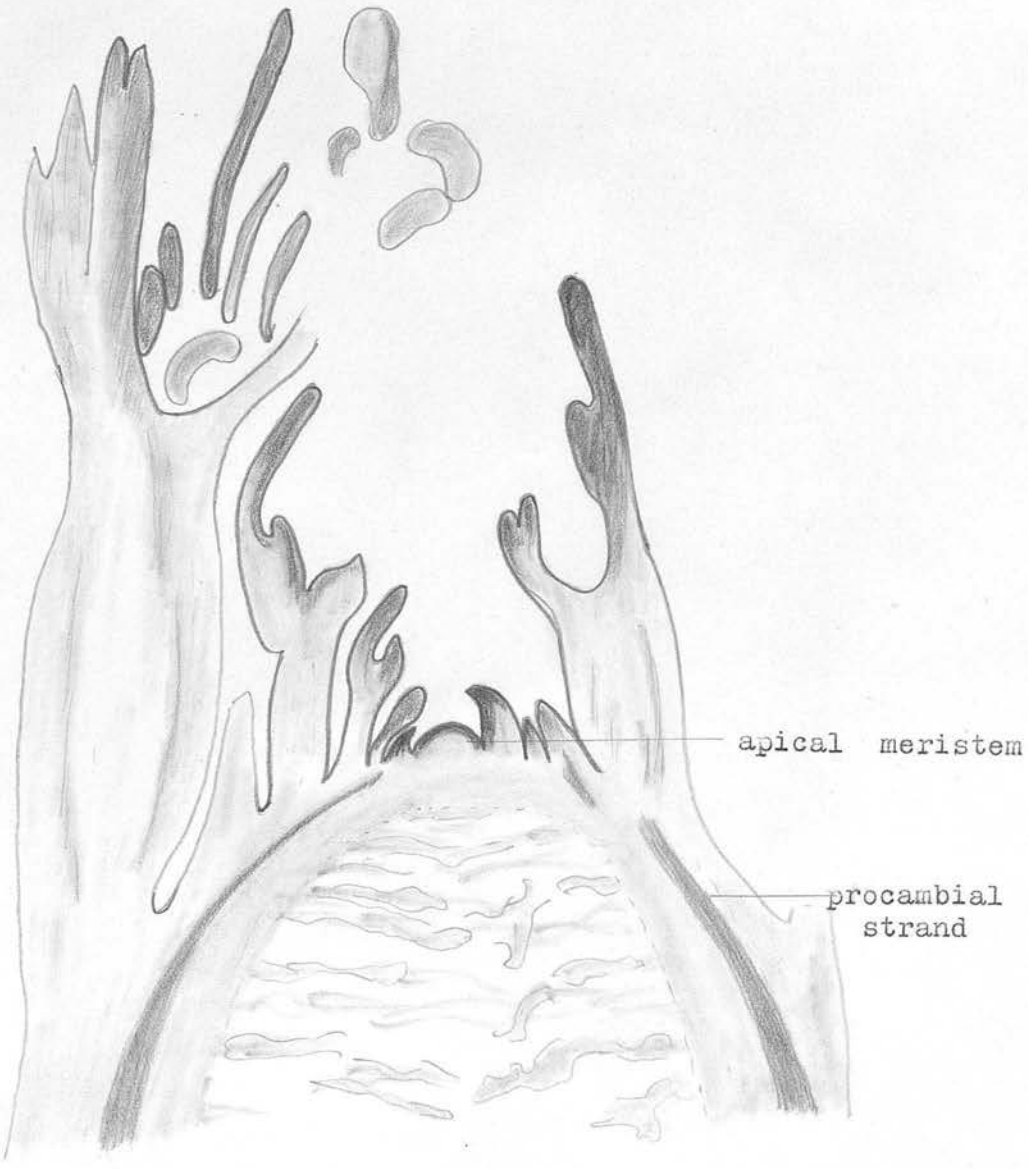


Figure 32.
DELPHINIUM. L.S. Apex. x 35.

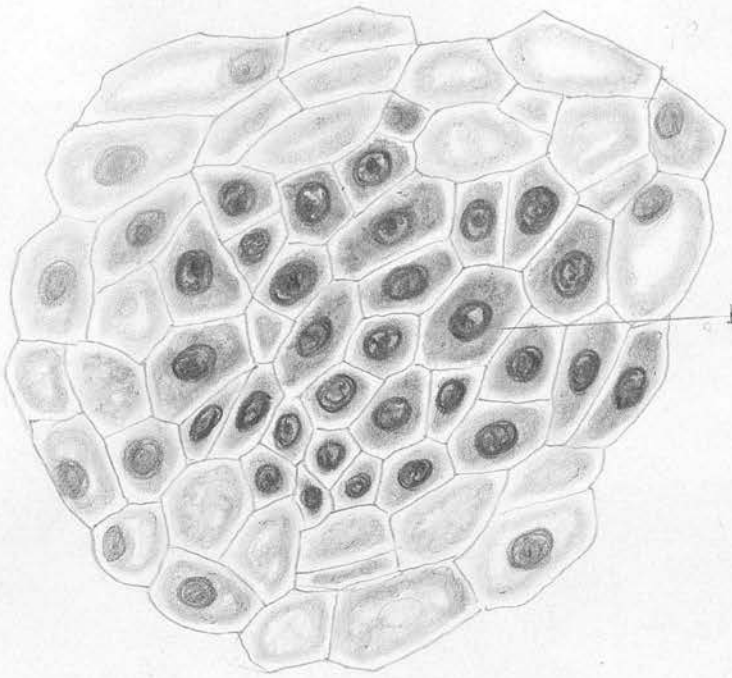


Figure 33. x 1000.
(130μ)

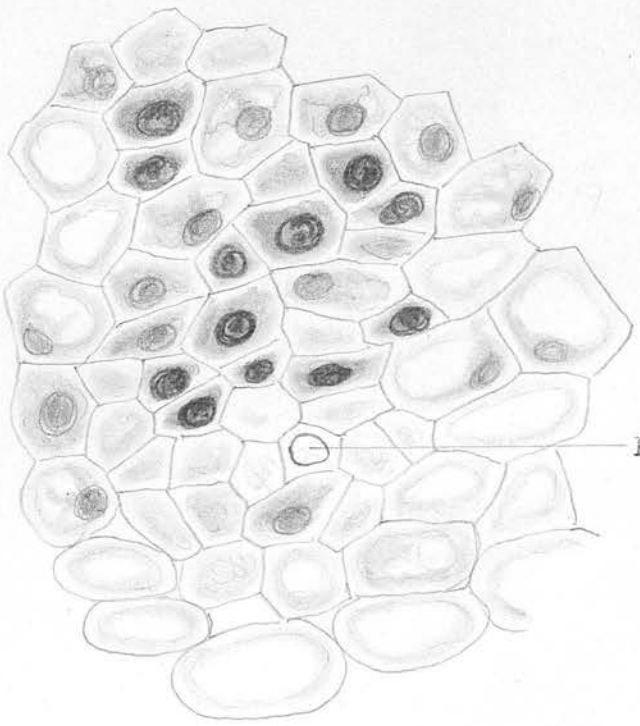
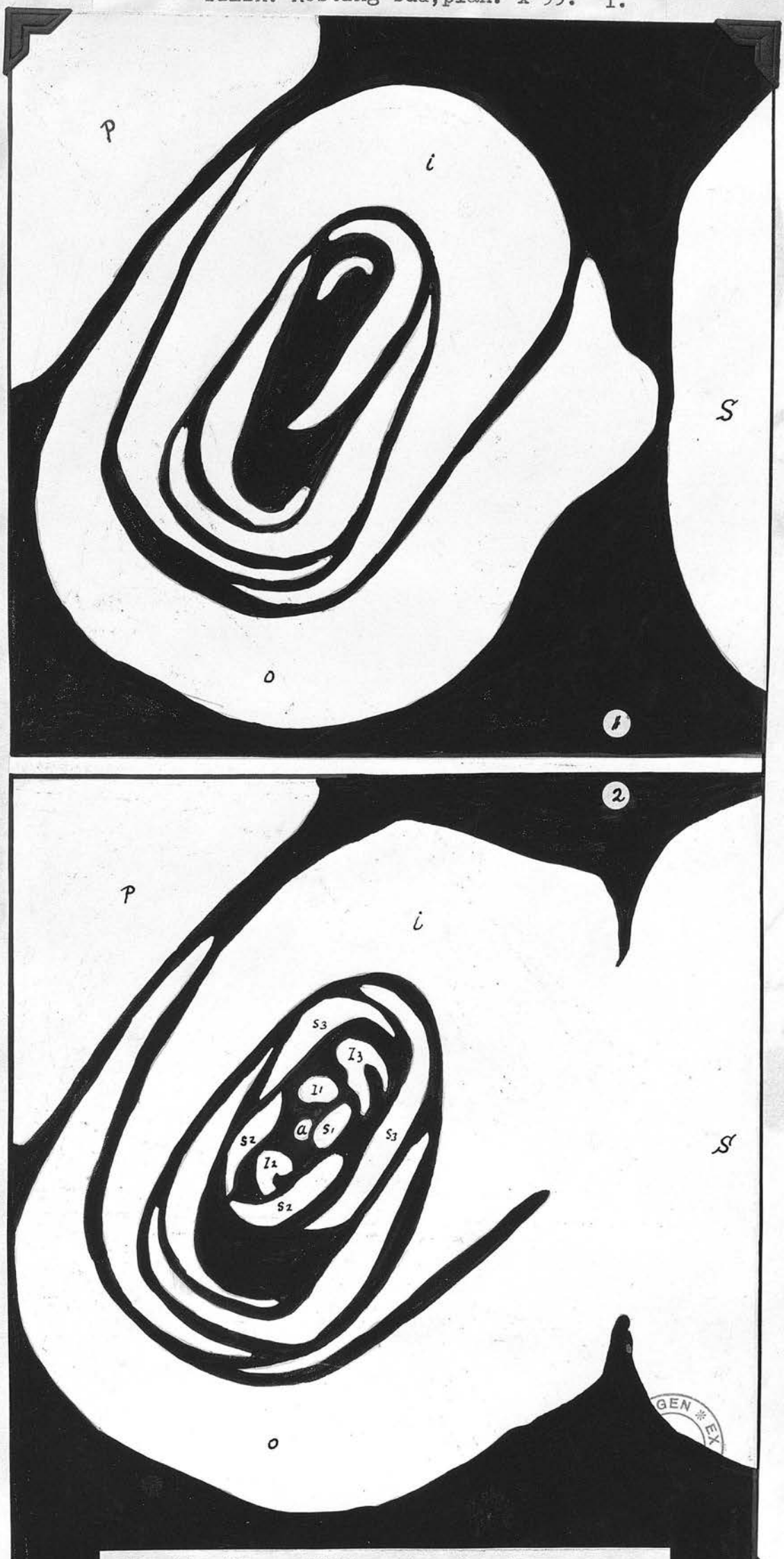


Figure 34. x 1000.
(180μ)

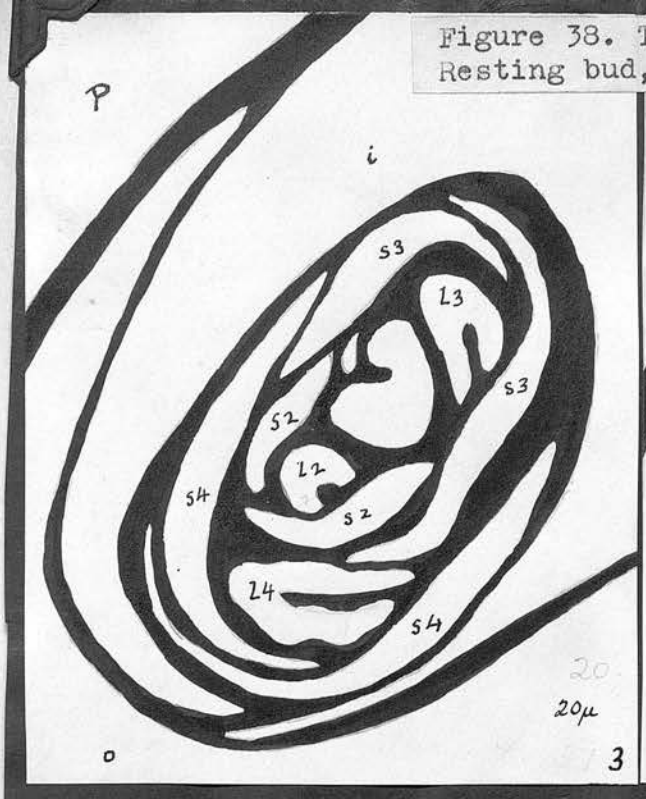
DELPHINIUM.

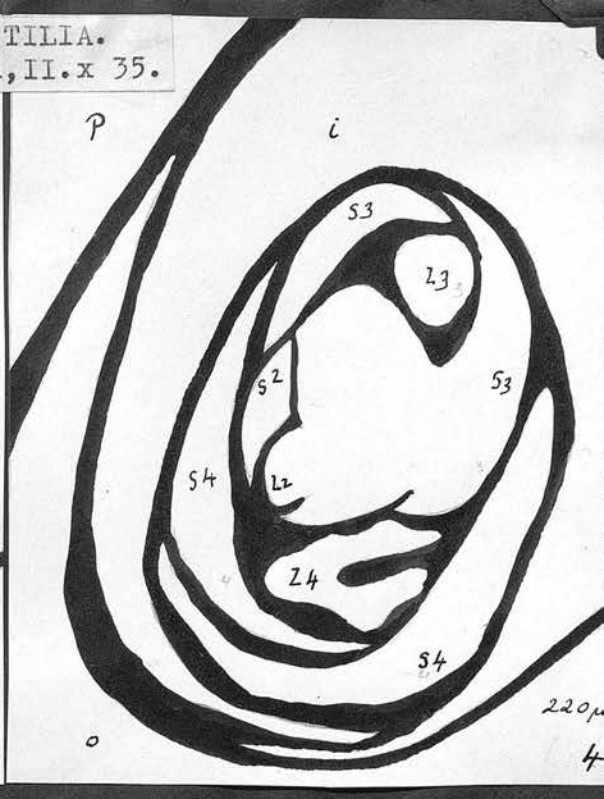
Figure 37.
TILIA. Resting bud, plan. x 35. I.

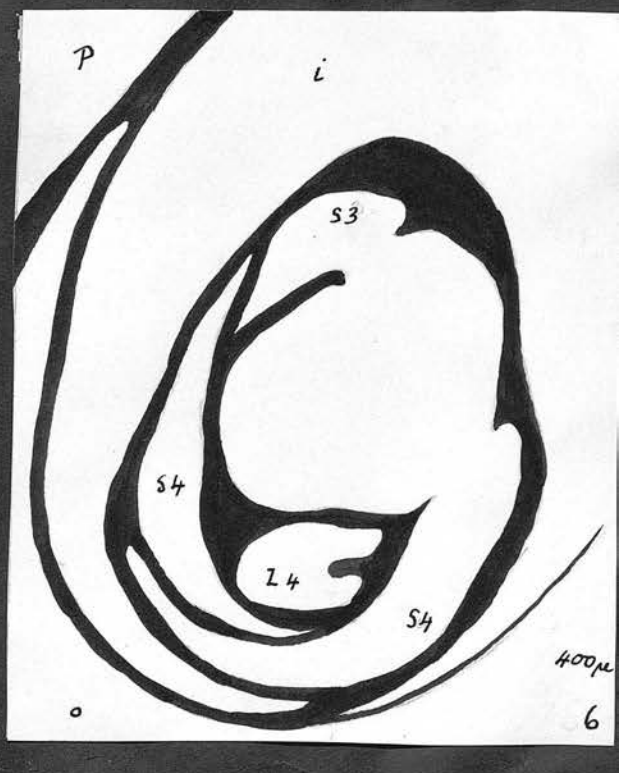


P, subtending petiole. S, main stem.
o, outer bud scale. i, inner bud scale.
l, leaf. s, stipule. (same symbols Fig. 38).

Figure 38. TILIA.
Resting bud, II.x 35.







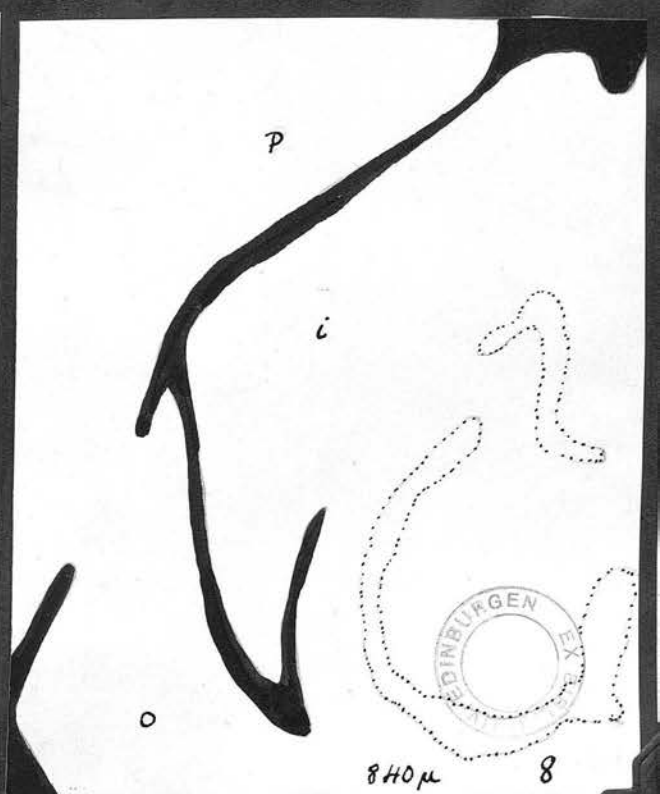
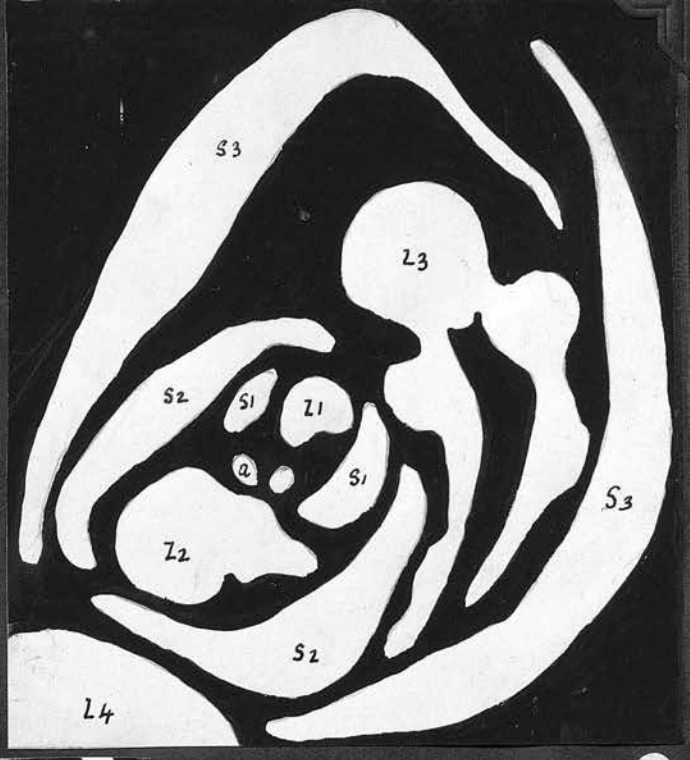


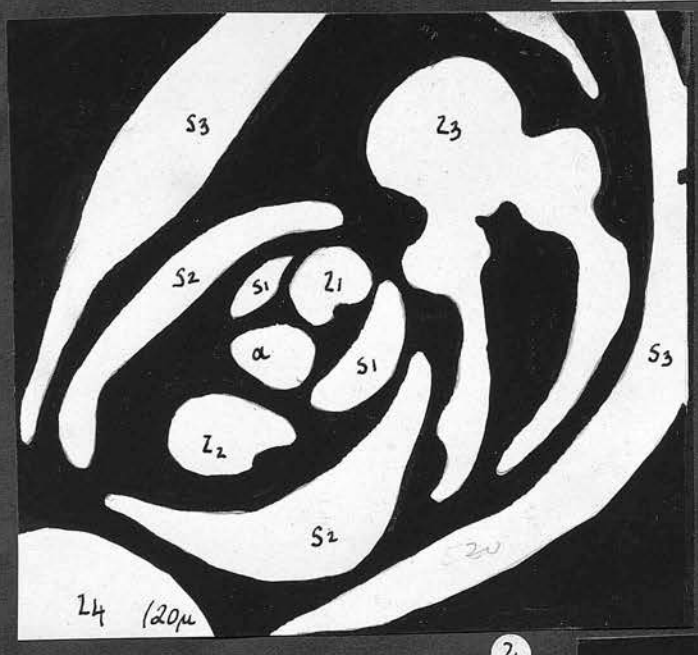
Figure 39. TILIA.
Opening bud. I. x 35.

l, leaf.
s, stipule.
a, stem apex.

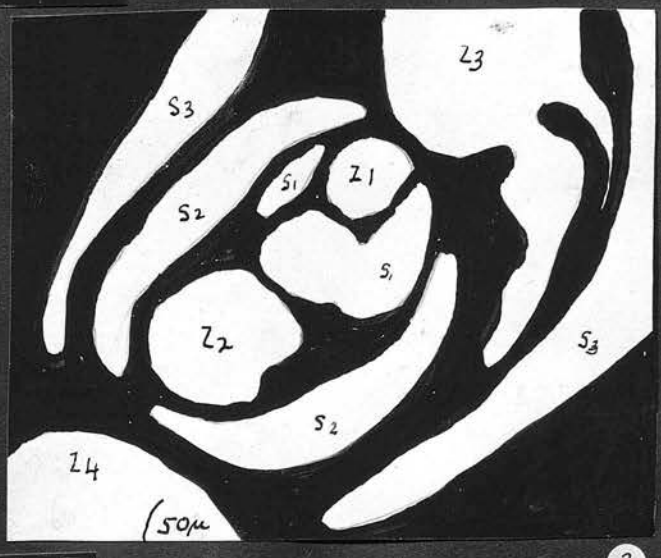
Orientation: in all figures adaxial side is towards lower right corner of figure.



1



2

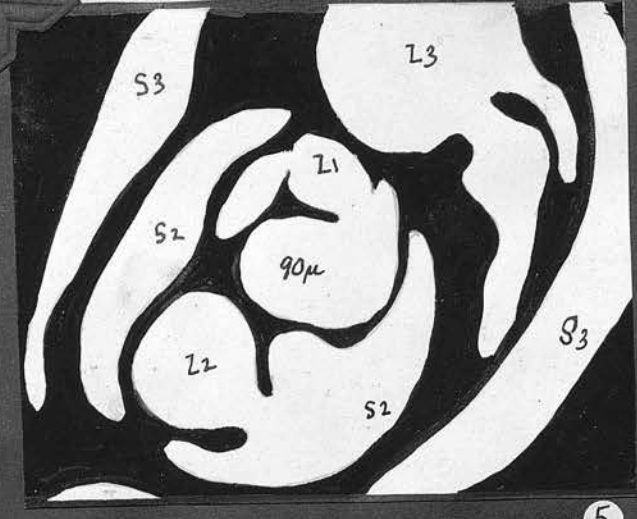


3

4

Figure 40. TILIA.
Opening bud II.
x 35.

Symbols and orientation as in Fig. 39



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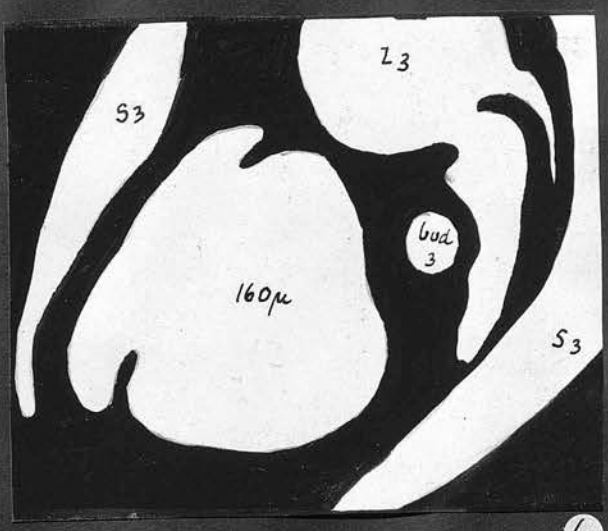

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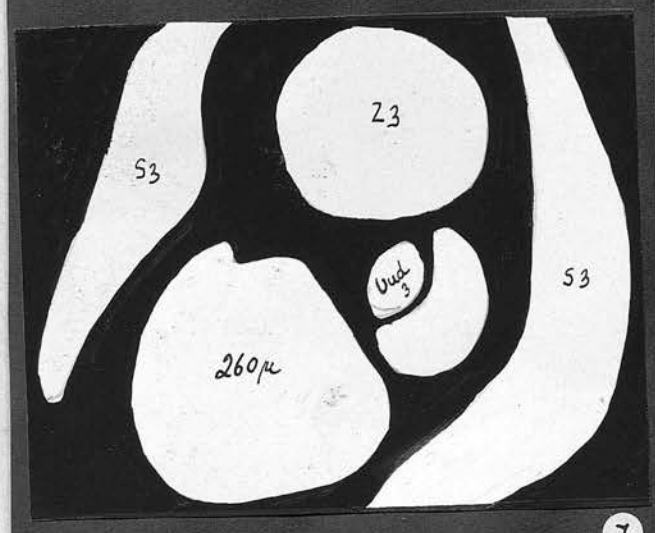
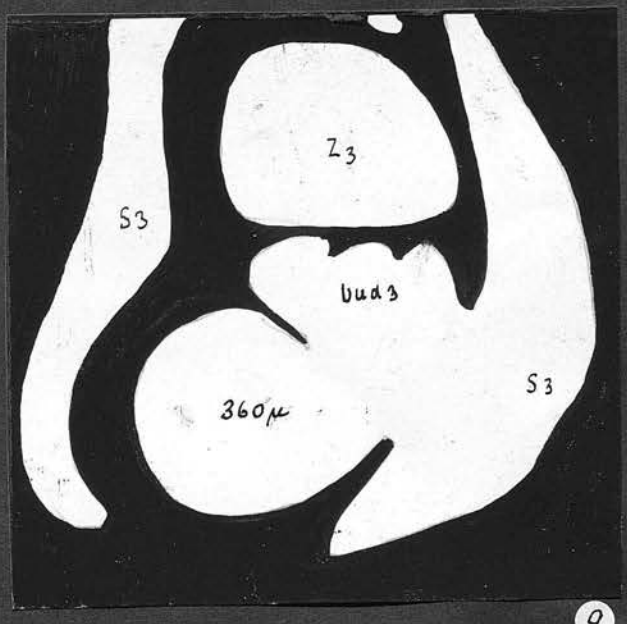


7

8



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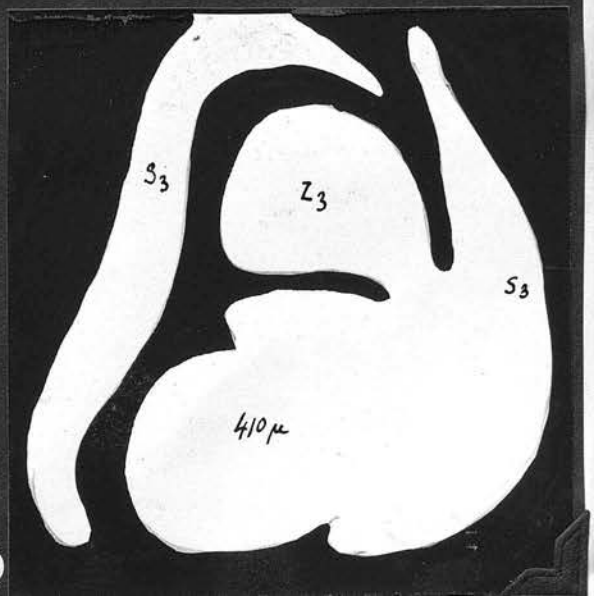

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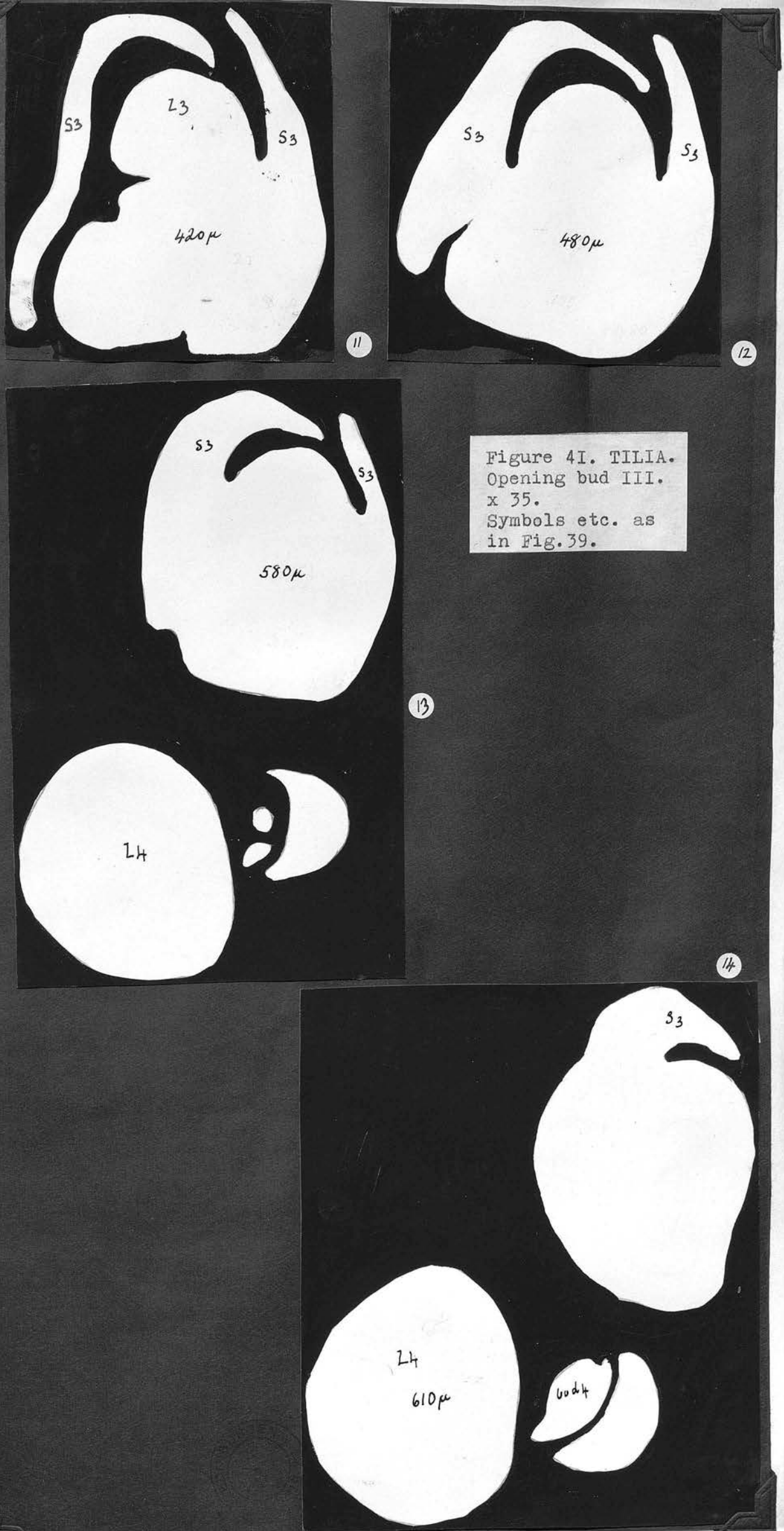
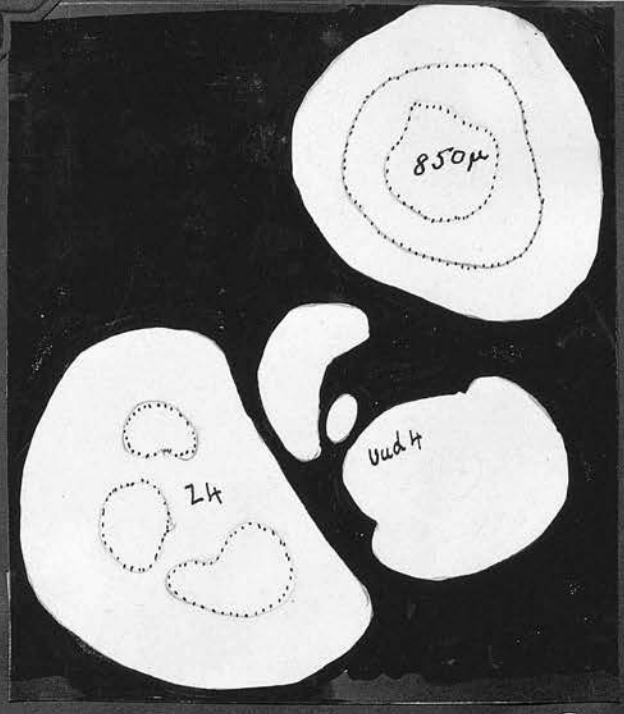
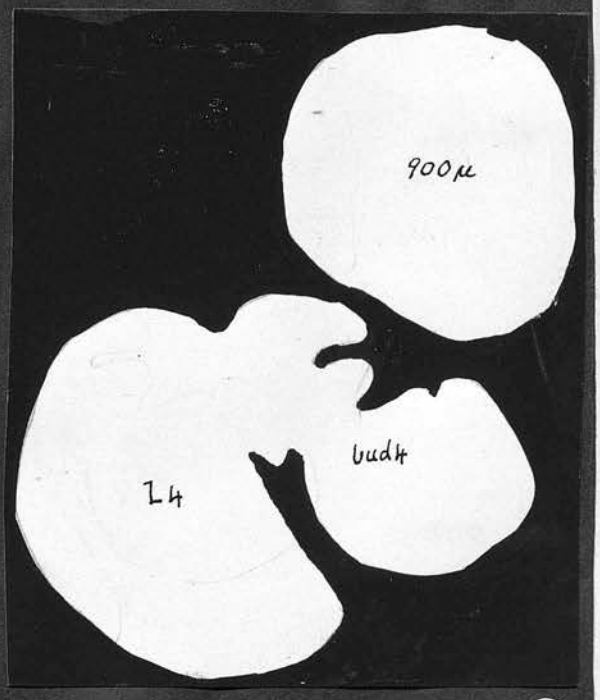


Figure 4I. TILIA.
Opening bud III.
x 35.
Symbols etc. as
in Fig.39.

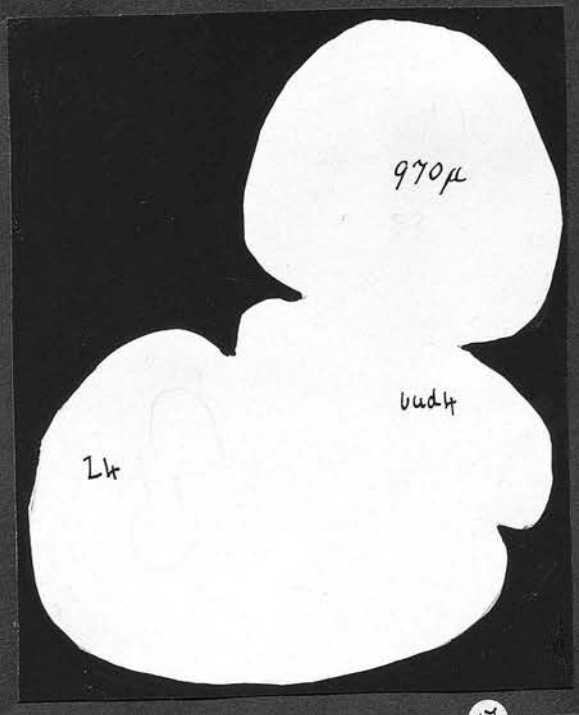


15

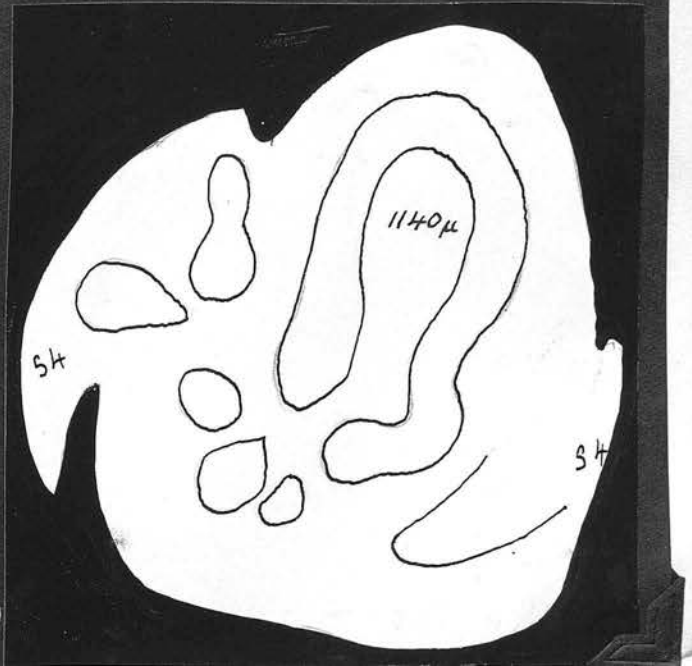
Figure 42. TILIA.
Opening bud. IV. x 35.
Symbols etc. as in
Fig. 39.



16



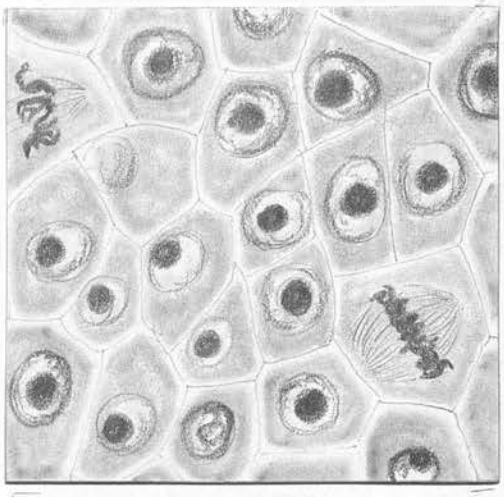
17



18

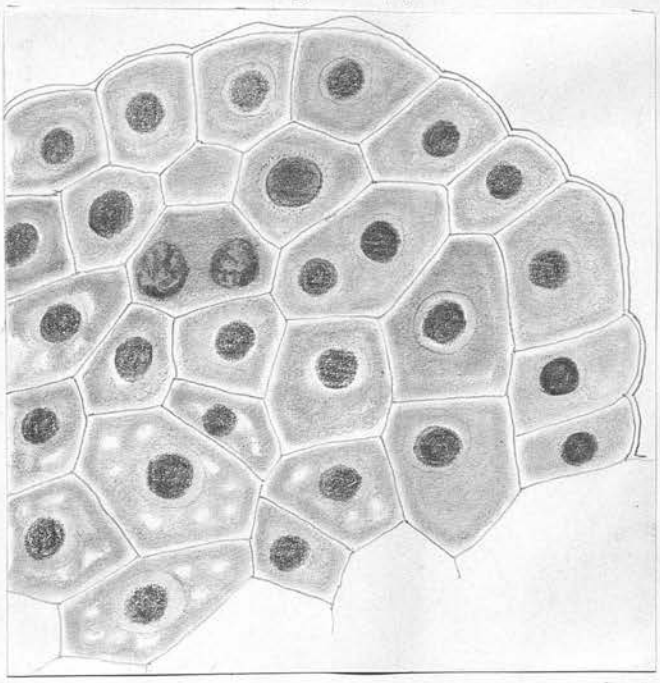
TILIA. x 1200.

Figure 43.



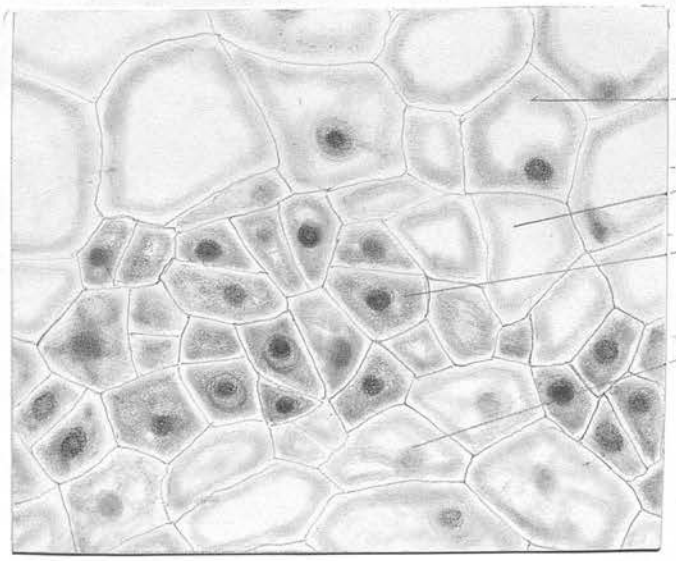
Apical meristem
(Bouin fixation).

Figure 44.



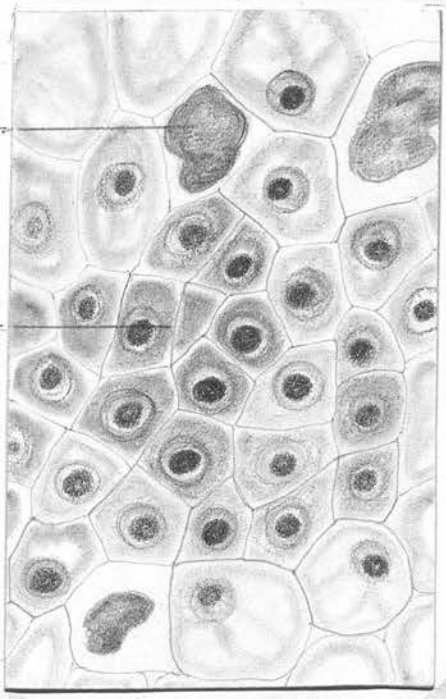
Leaf primordium (Zirkle fixation).

Figure 45. (80μ)



Zirkle fixation

Figure 46. (100μ)



Bouin fixation

TILIA. x 1200.

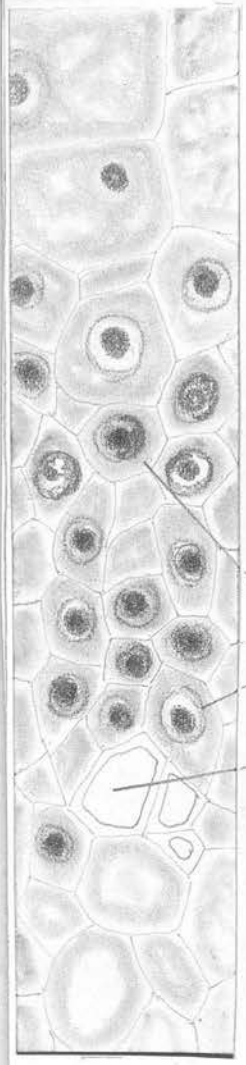


Figure 47.
(380μ)

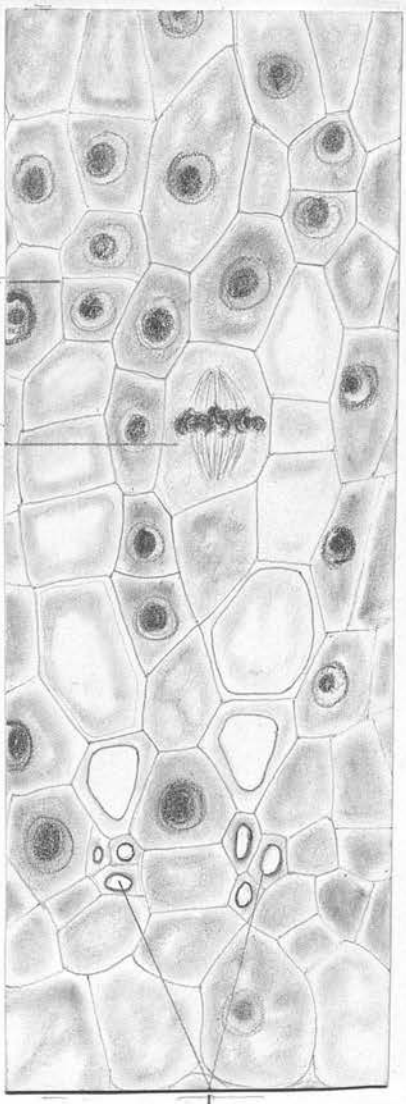


Figure 48. (500μ)

Figure 49. (950μ).

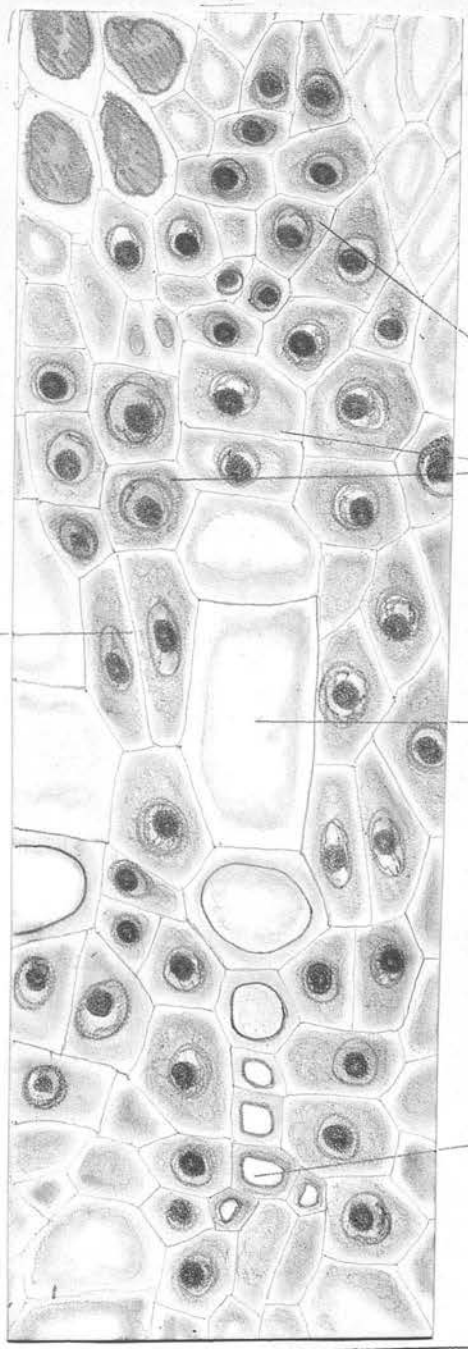
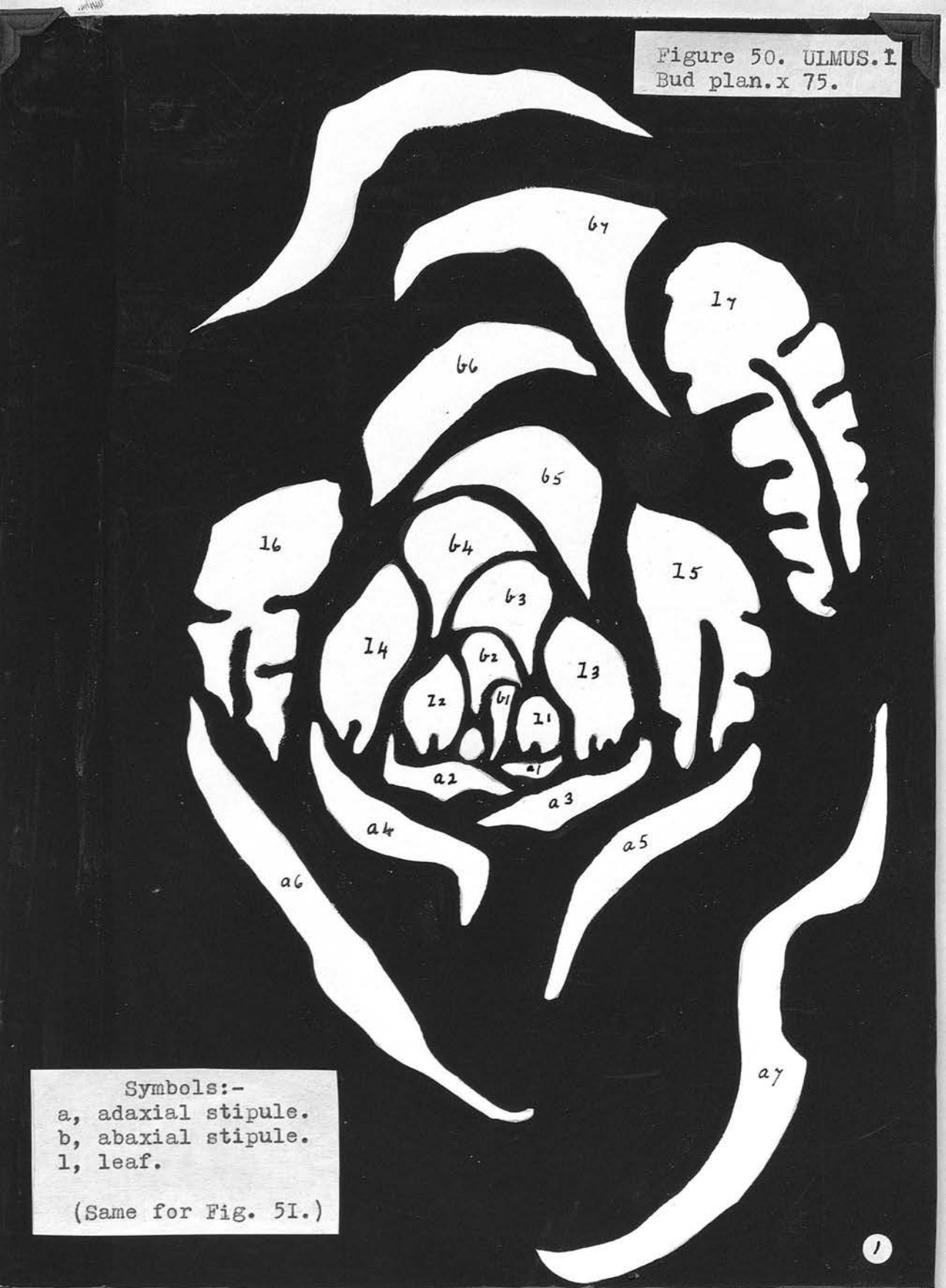
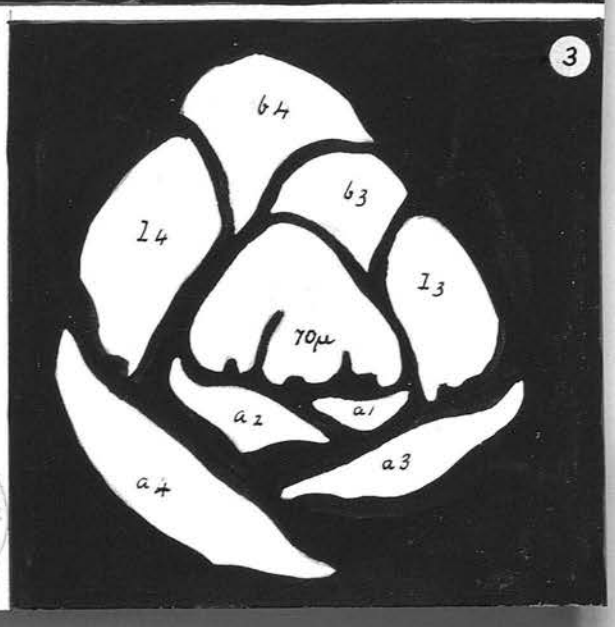


Figure 50. ULMUS. 1
Bud plan. x 75.



Symbols:-
a, adaxial stipule.
b, abaxial stipule.
l, leaf.

(Same for Fig. 51.)



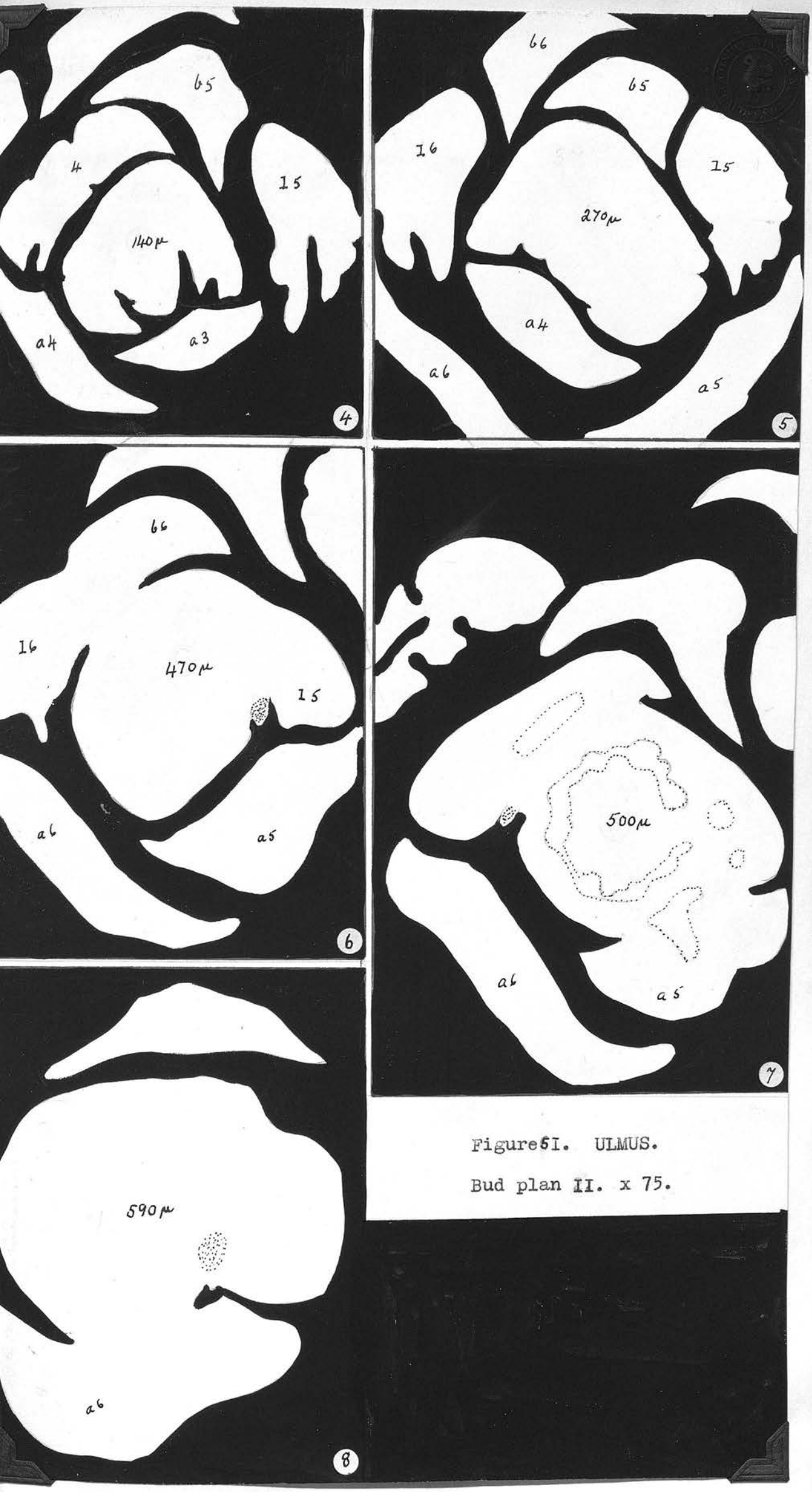
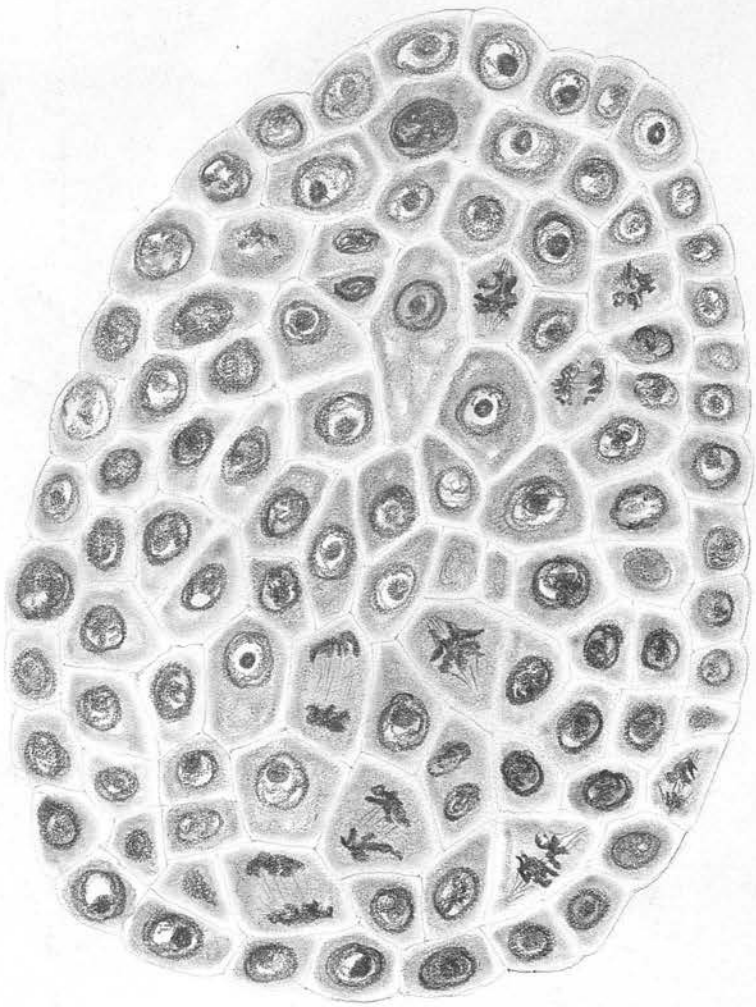


Figure 51. ULMUS.

Bud plan II. x 75.

ULMUS. x 1000.



apical
meristem.

Figure 52.

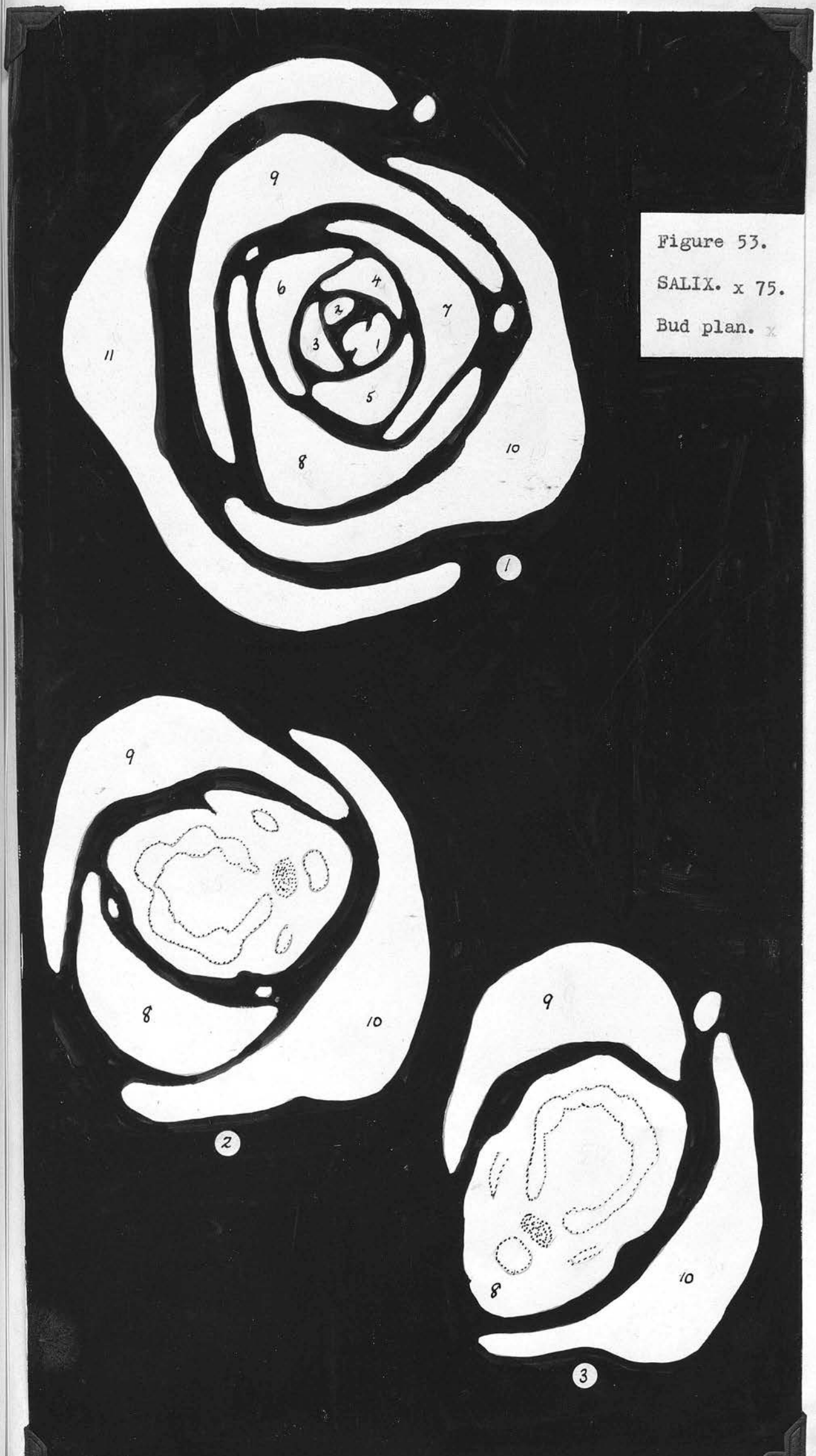


Figure 53.

SALIX. x 75.

Bud plan. x

Figure 54.
BETULA.
BUd plan. x 75.

Figure 55. (80μ)

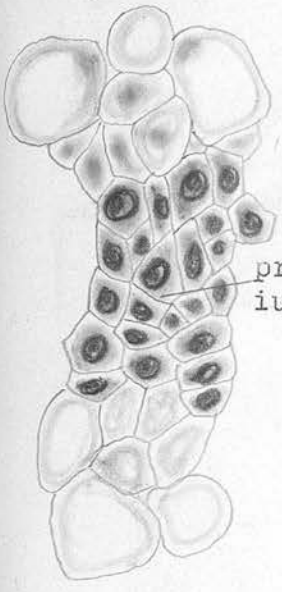


Figure 56. (220μ)

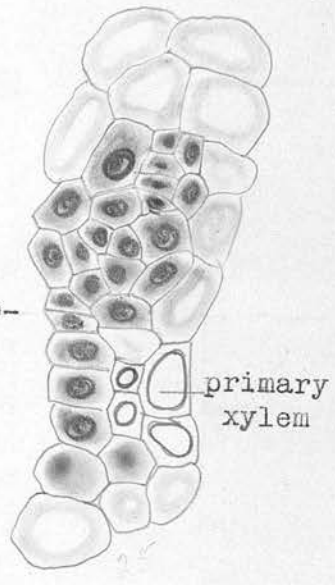
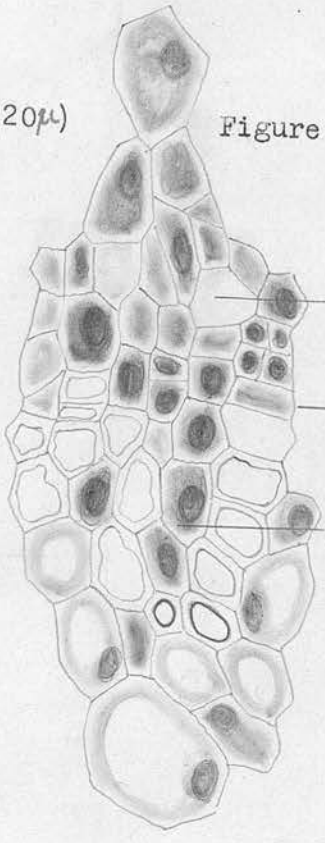


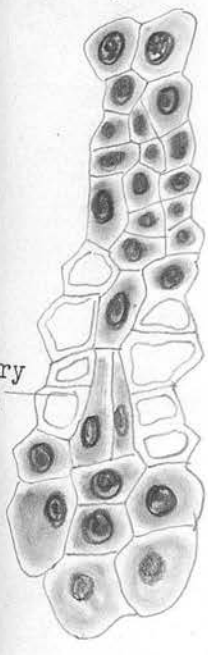
Figure 57. (270μ)



SALIX. x 1000.

Figure 59 (700μ)

Figure 58.
(500μ)



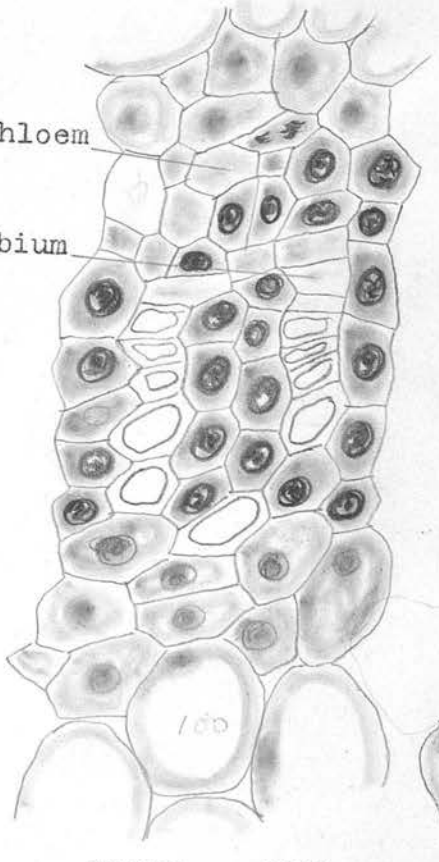
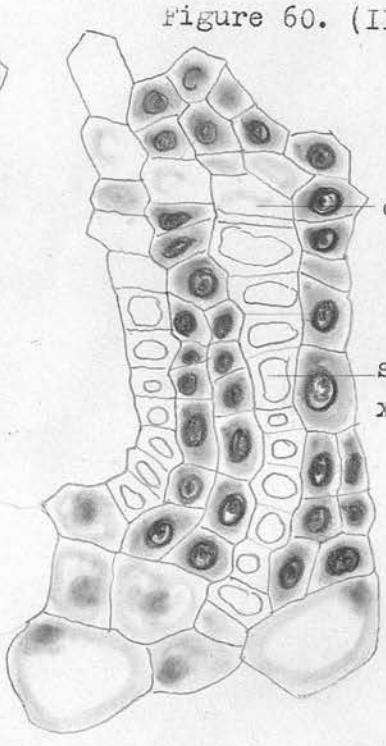


Figure 60. (1100μ)



ULMUS. x 1000.

ULMUS. x 1000.

BETULA. x 1000.

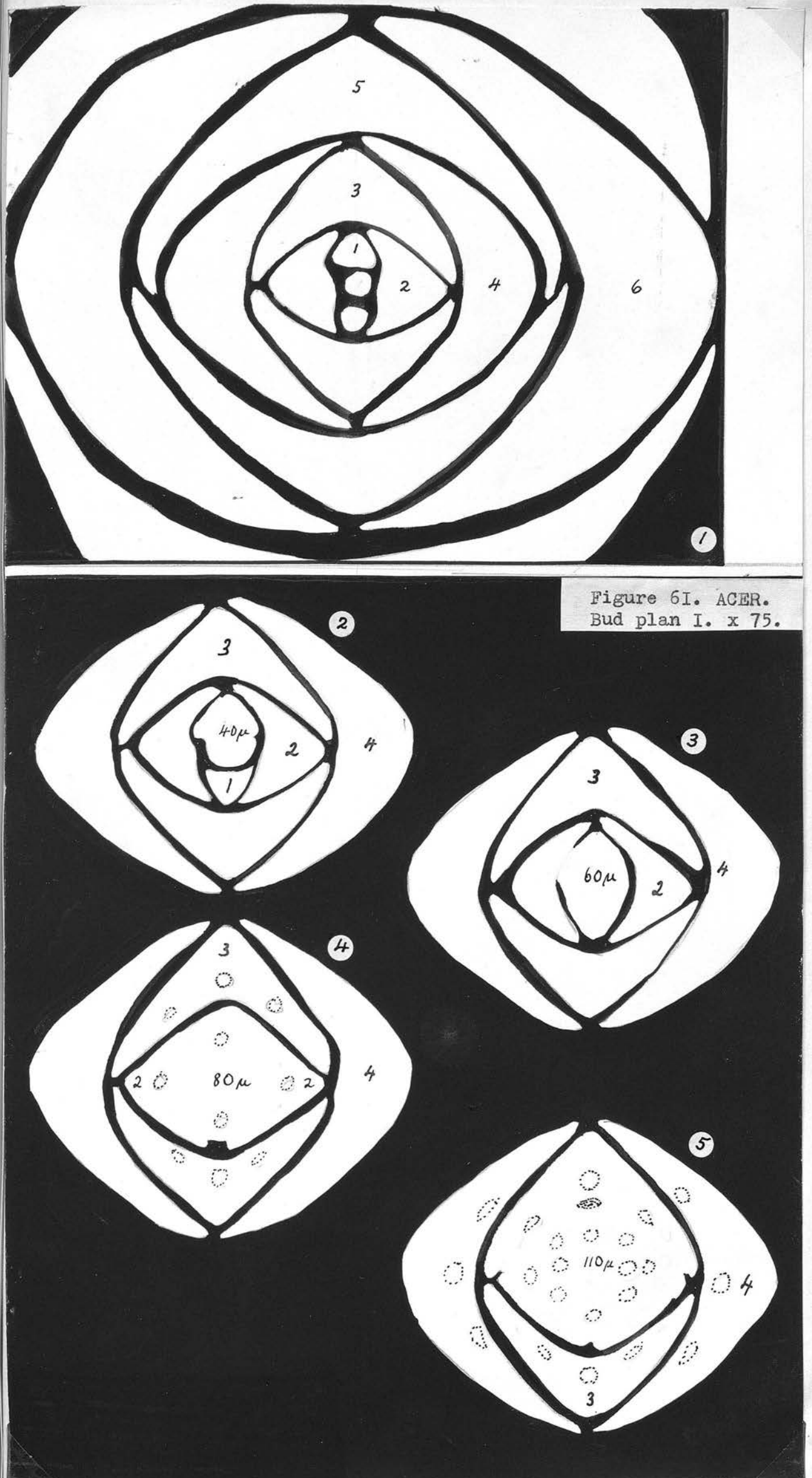


Figure 6I. ACER.
Bud plan I. x 75.

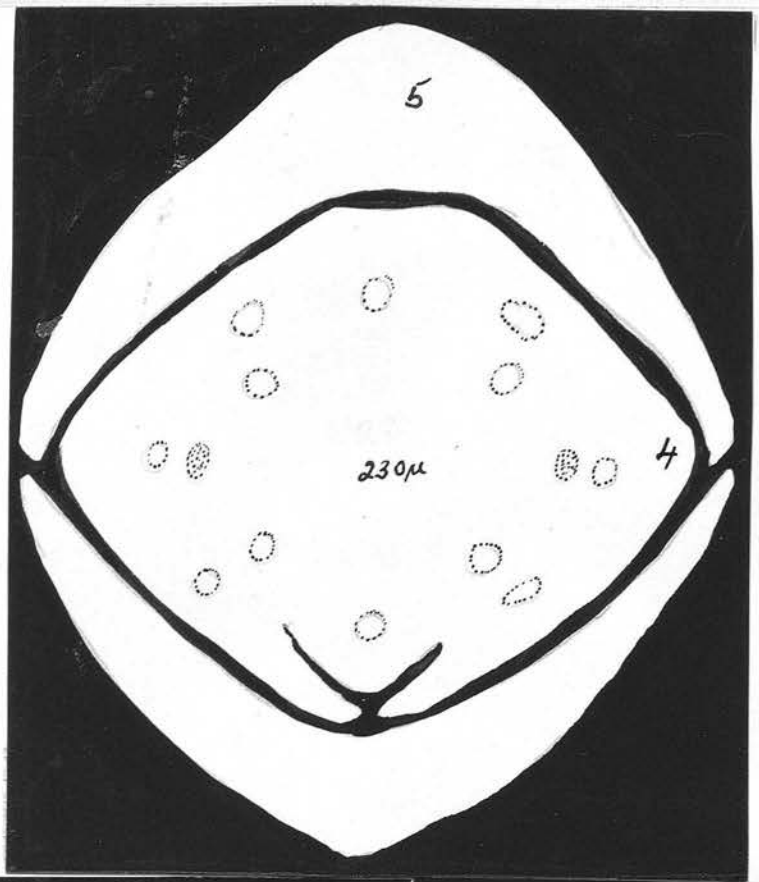
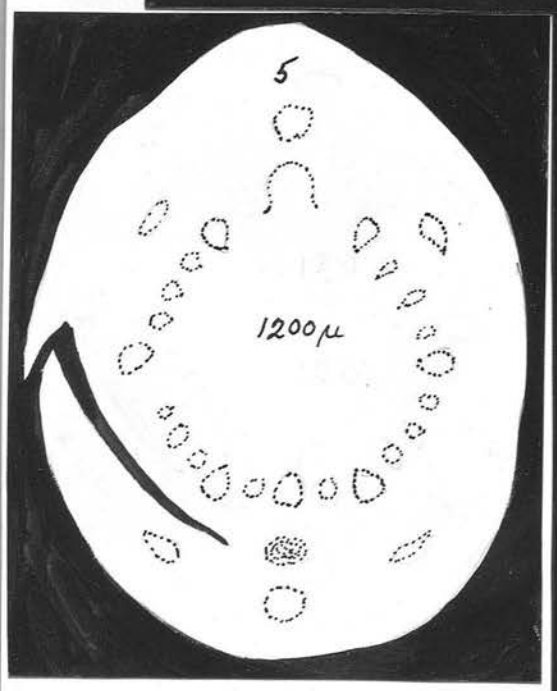
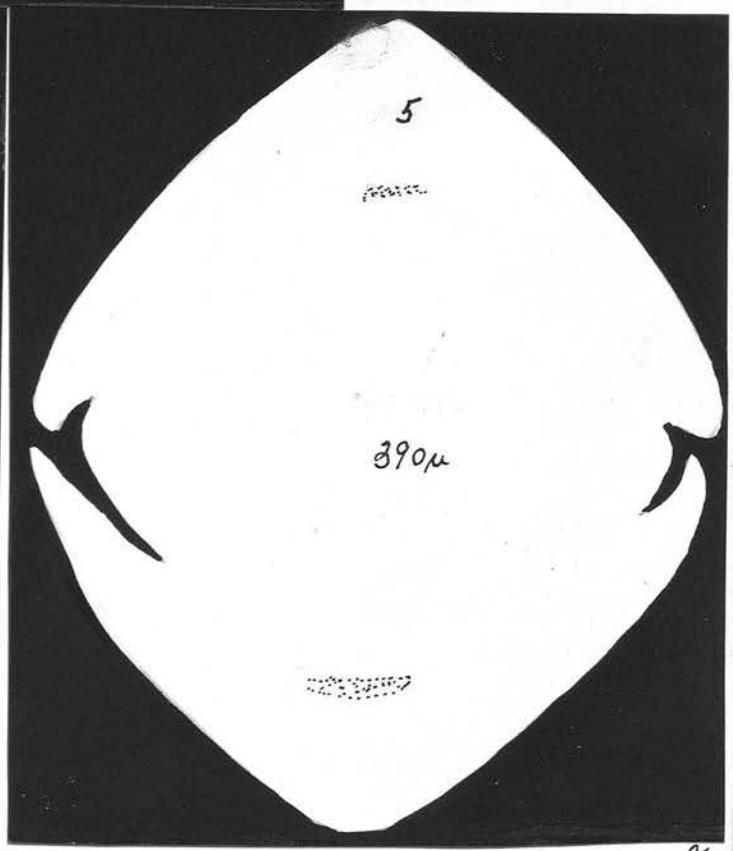


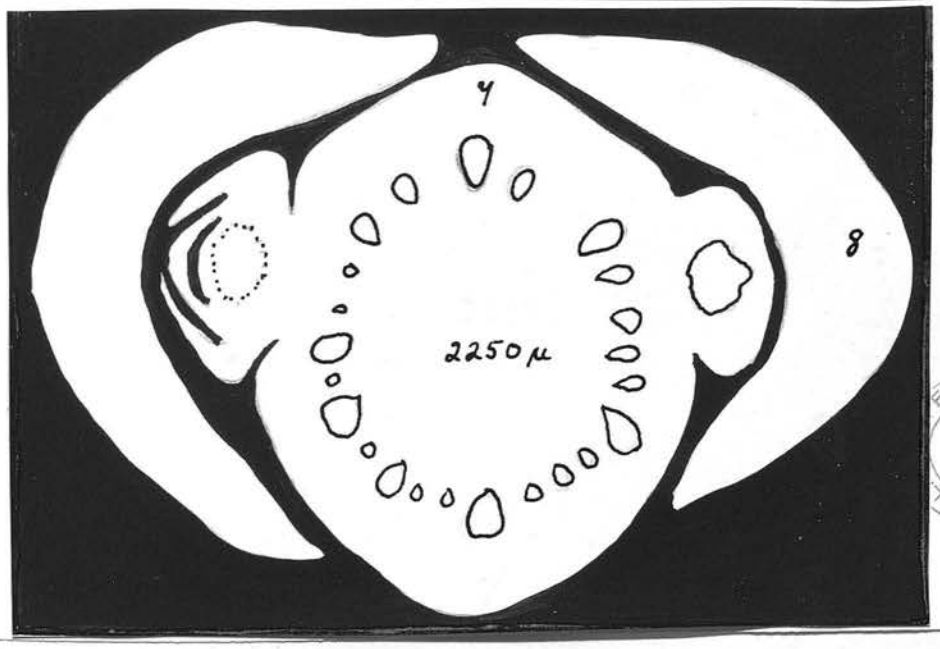
Figure 62.

ACER.

Bud plan II.x 75.

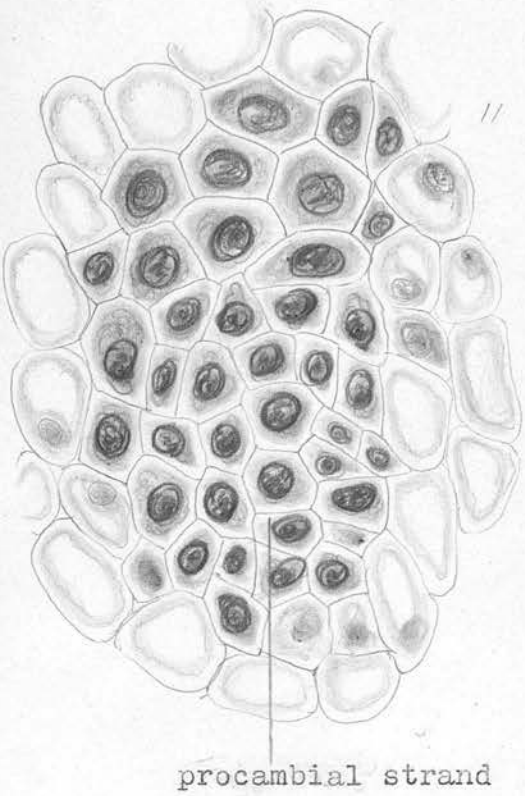






ACER. X 1000.

Figure 63. (90μ)



procambial strand

Figure 64. (230μ)

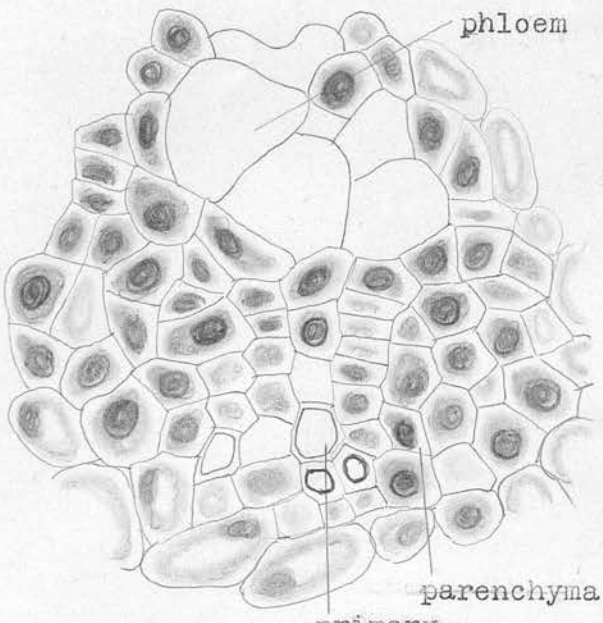


Figure 65.
(400μ)

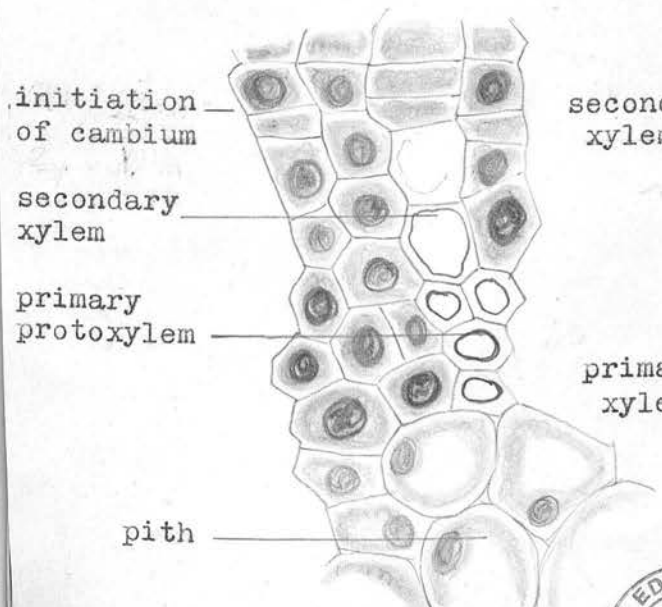


Figure 66. (1200μ)

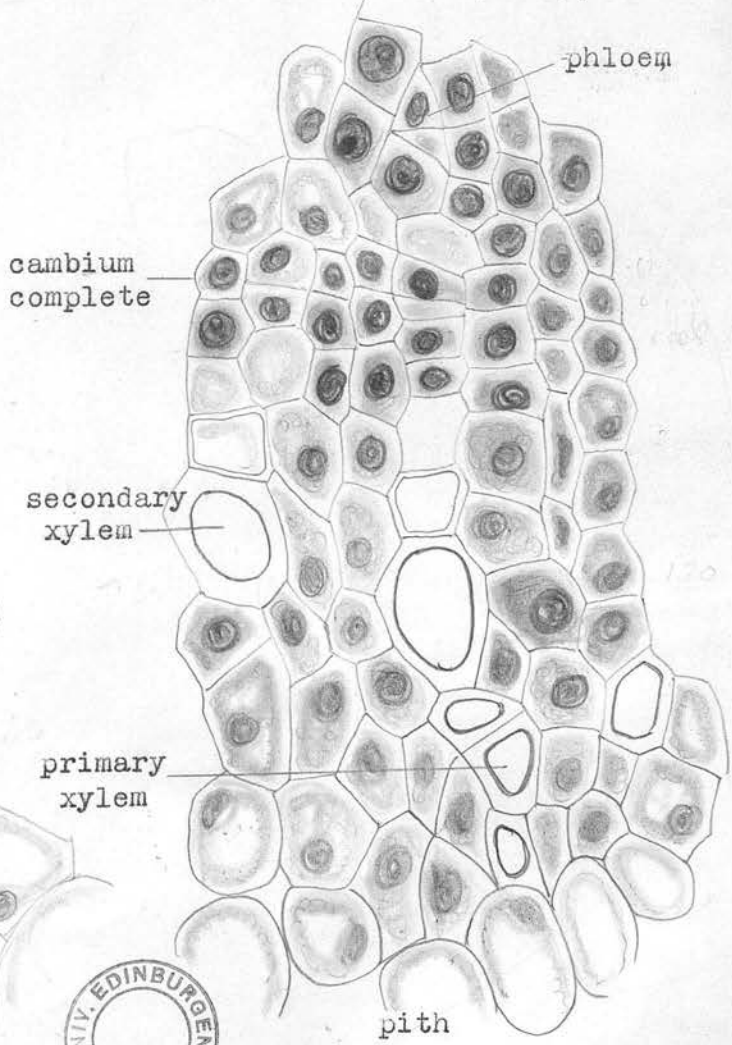


Figure 67.

Acer: plan of internode where interfascicular cambium begins. x 75.

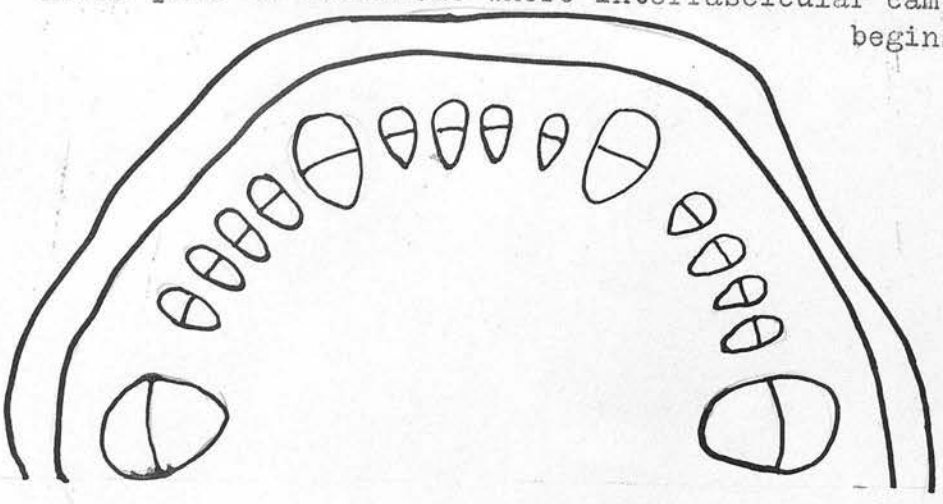


Figure 68. origin of interfascicular cambium.

Figure 69.

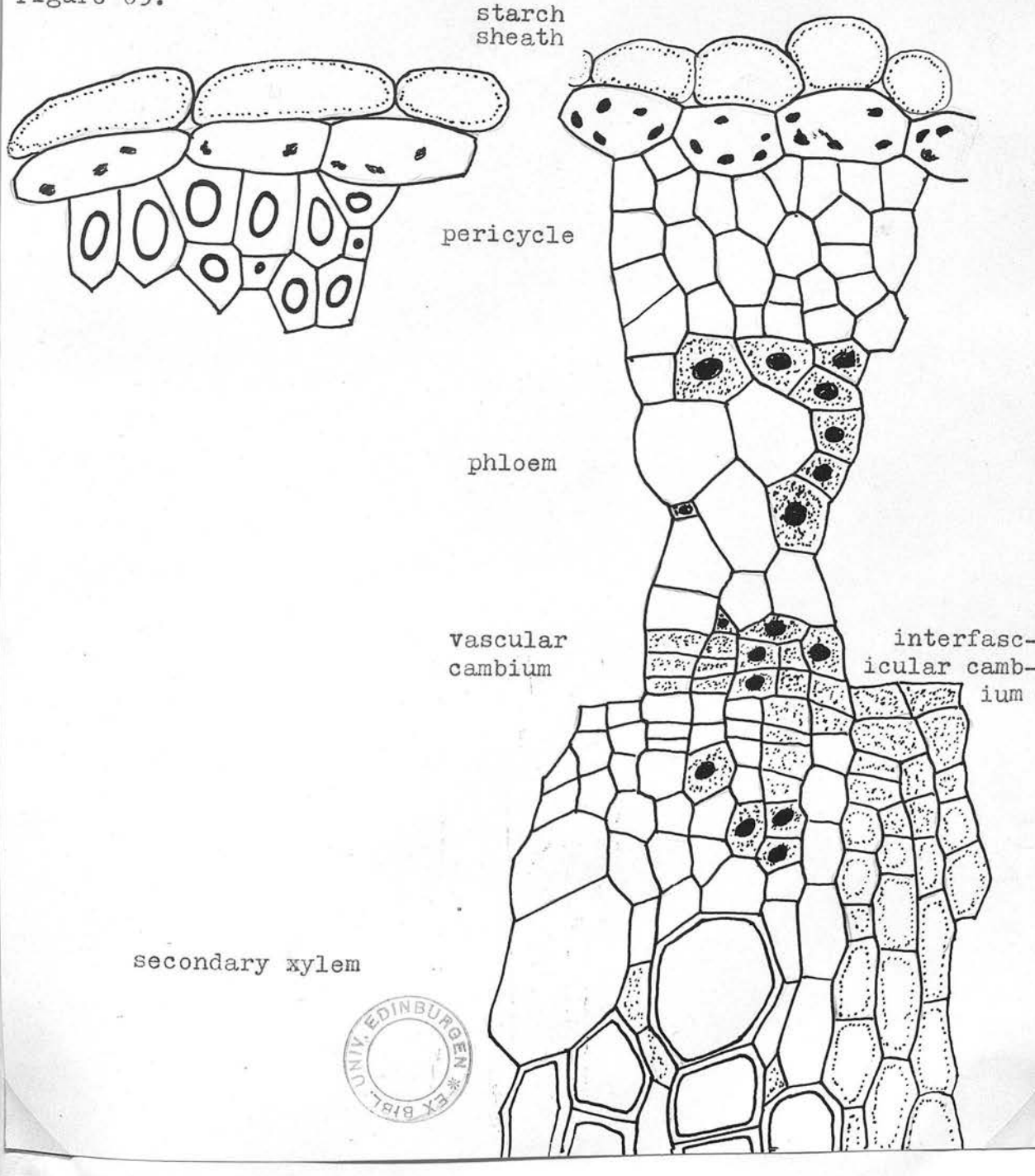
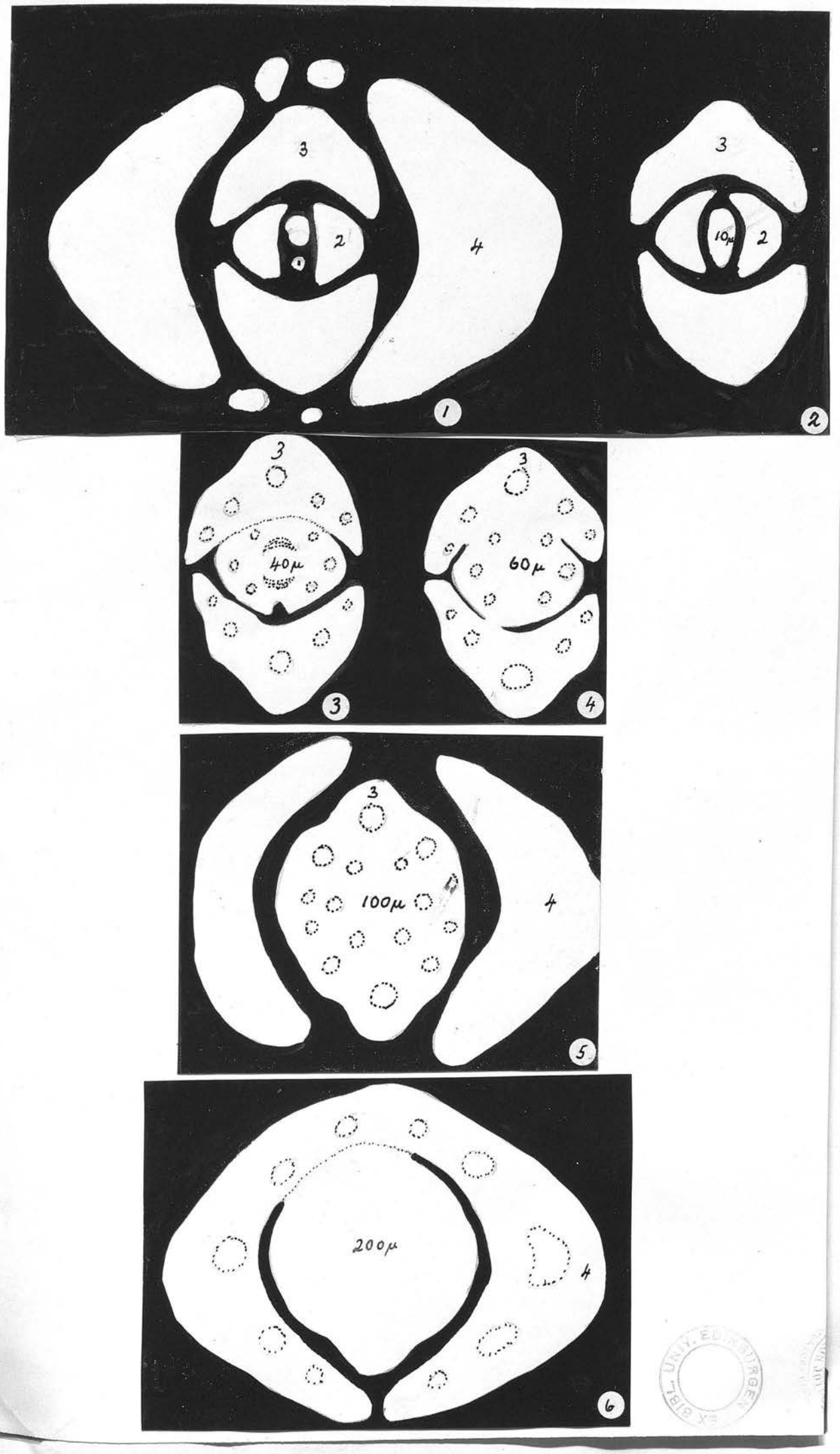


Figure 70.

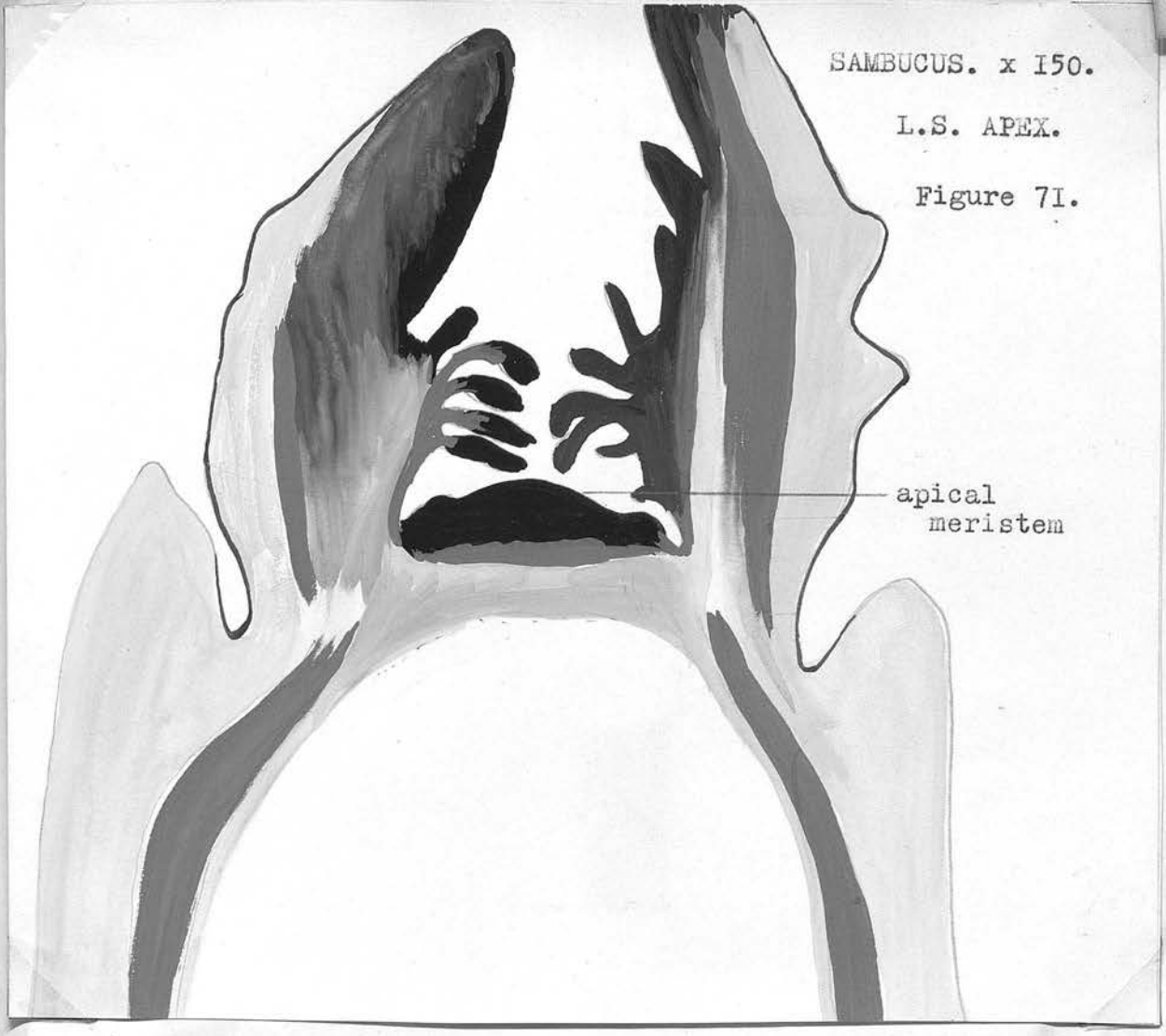
SAMBUCUS. Bud plan. x 75.



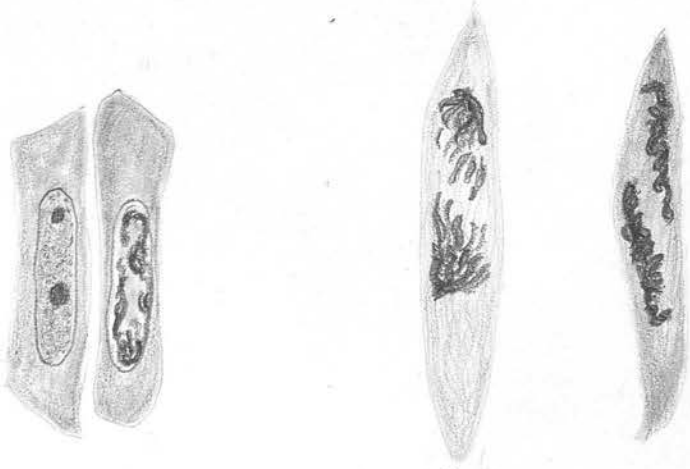
SAMBUCUS. x 150.

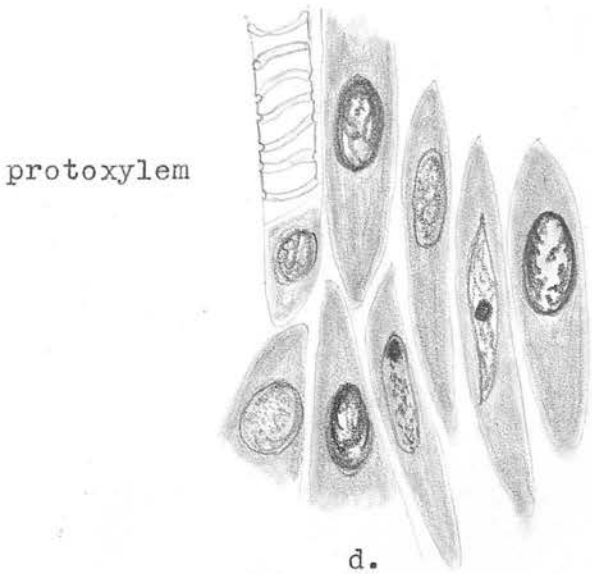
L.S. APEX.

Figure 71.



SAMBUCUS. x 1000. Details of procambium in longitudinal view.
Figure 72.





SAMBUCUS. X 1000.

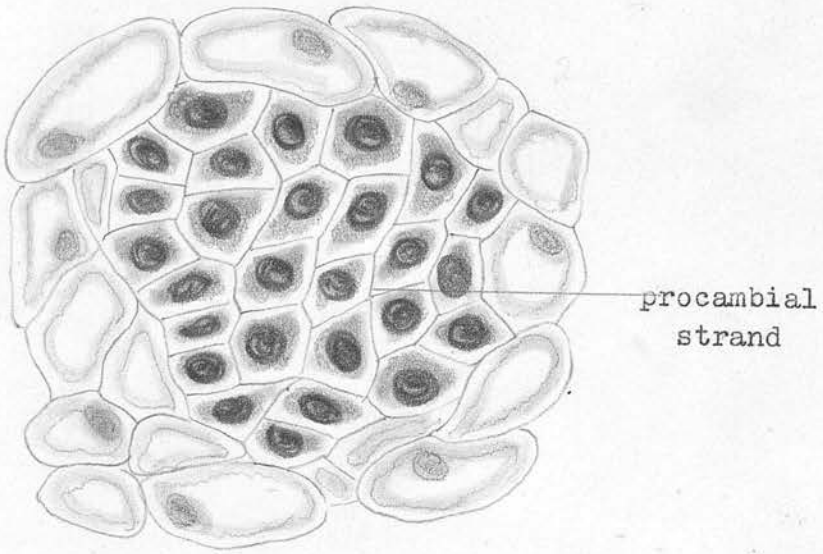


Figure 73. (40μ)

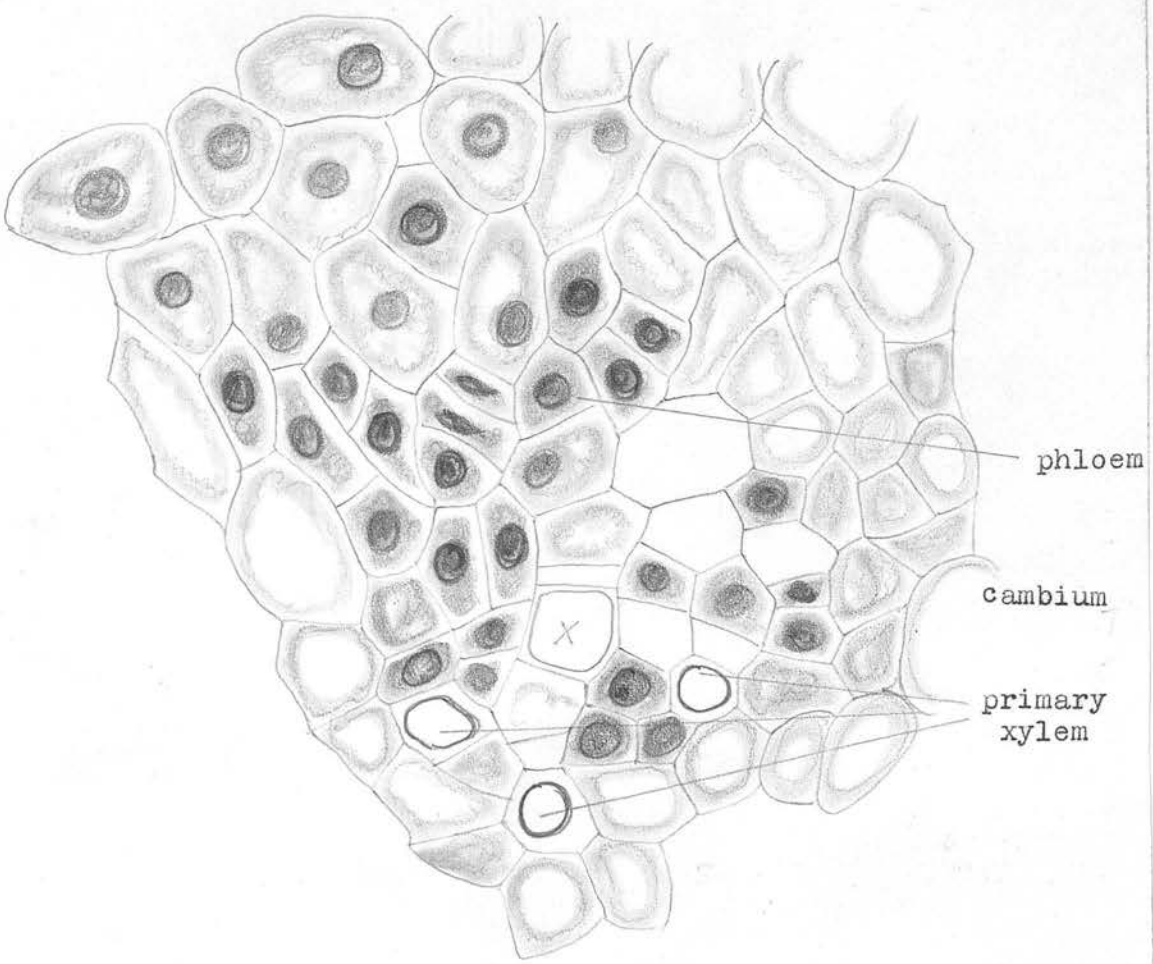


Figure 74. (170μ)

Sambucus.

Figure 75. x 75.

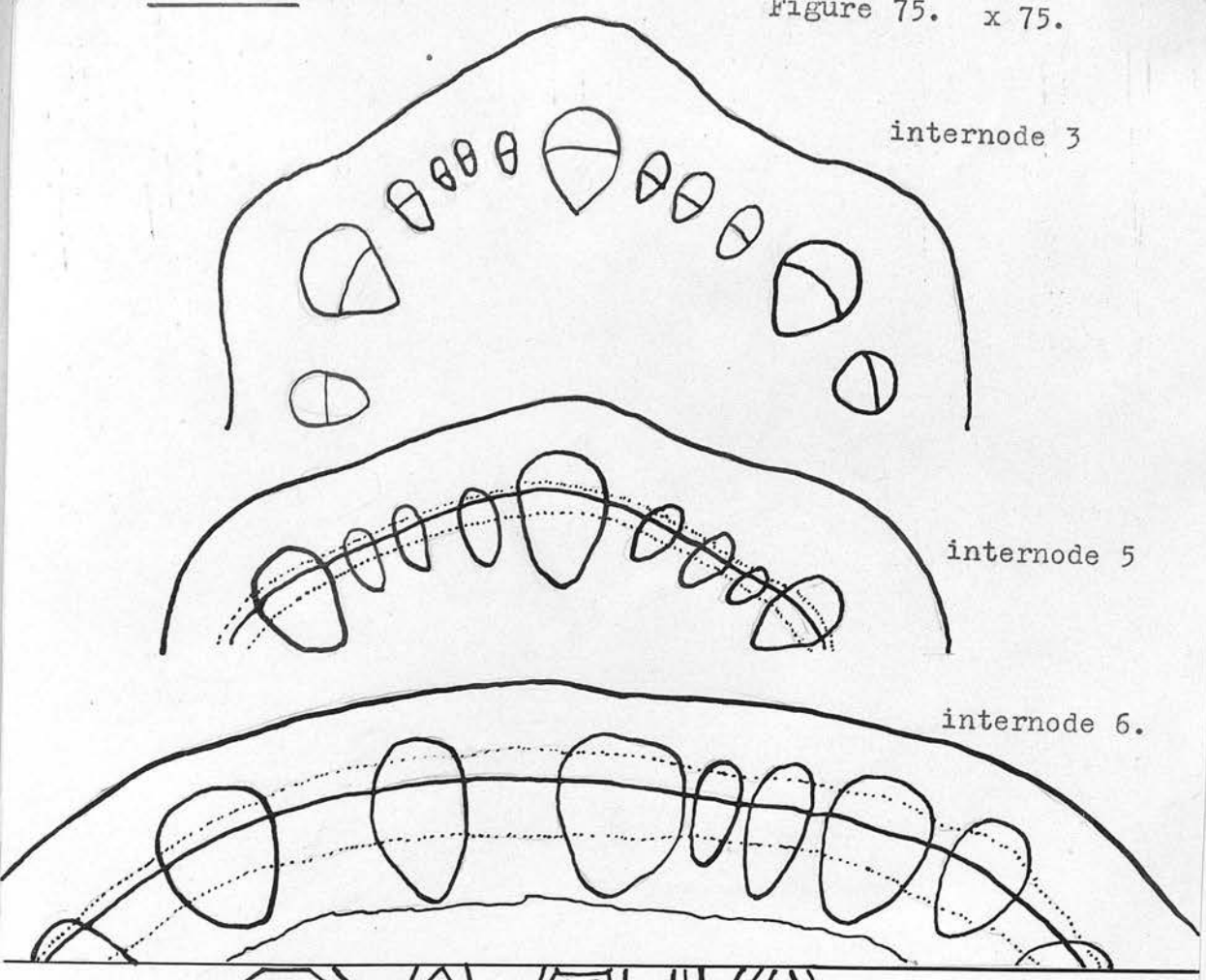


Figure 76. x 500

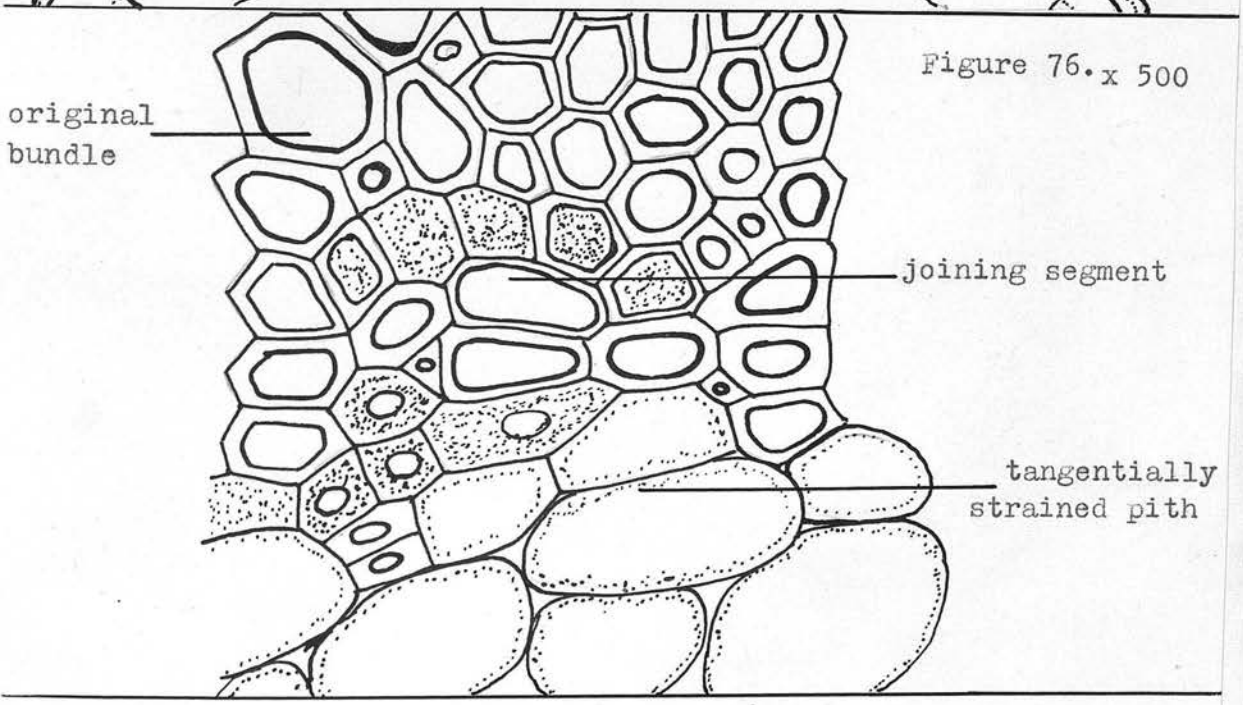
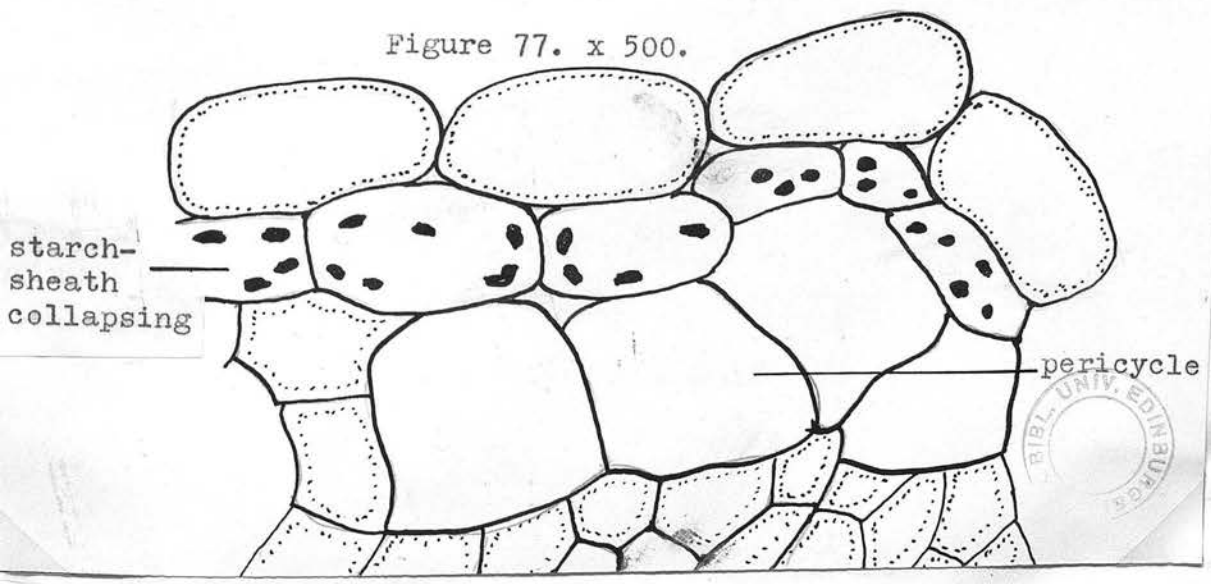
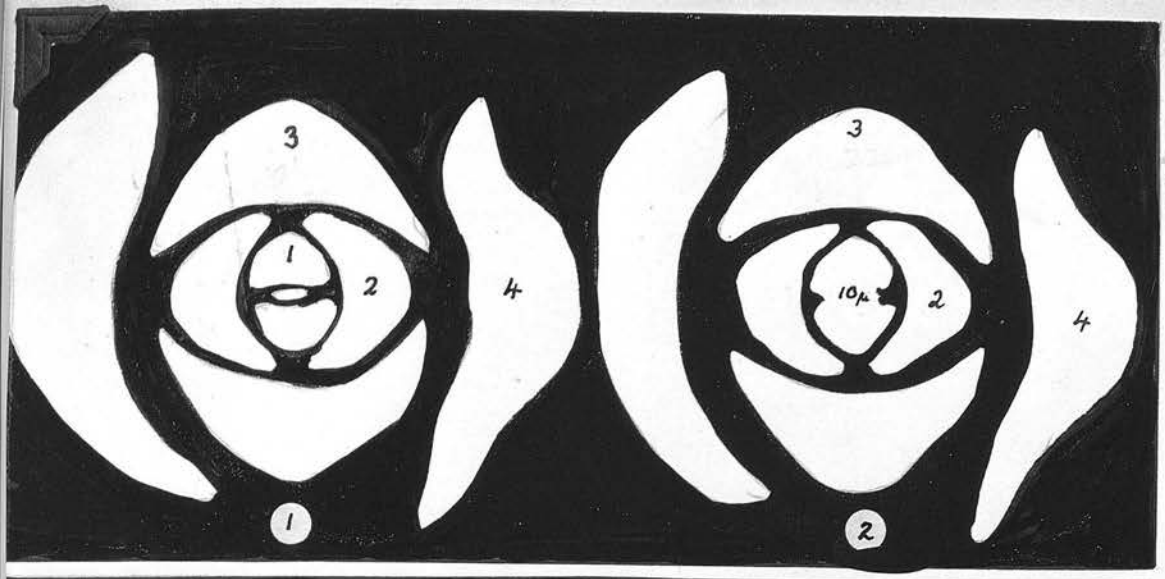
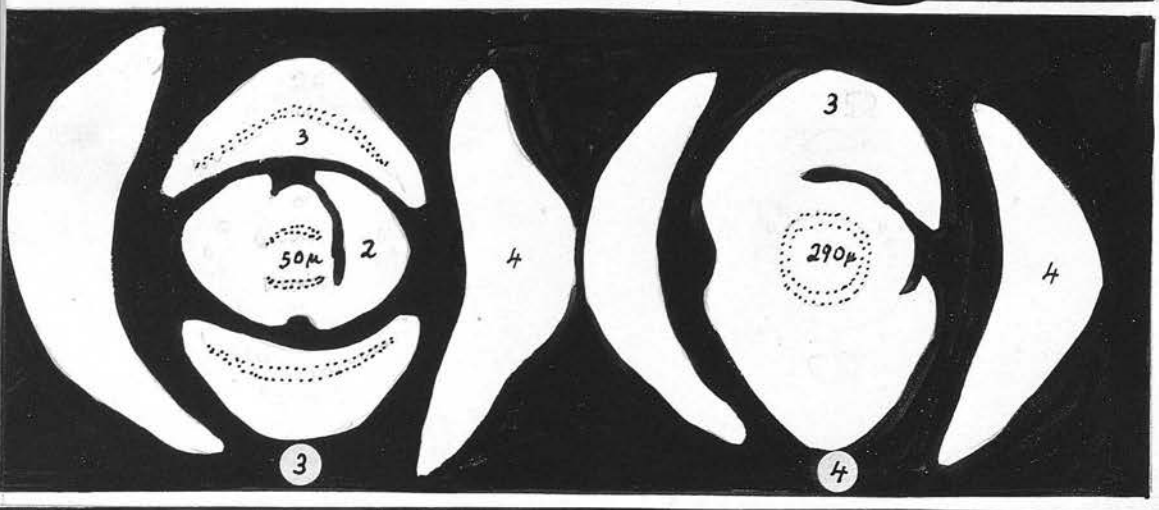
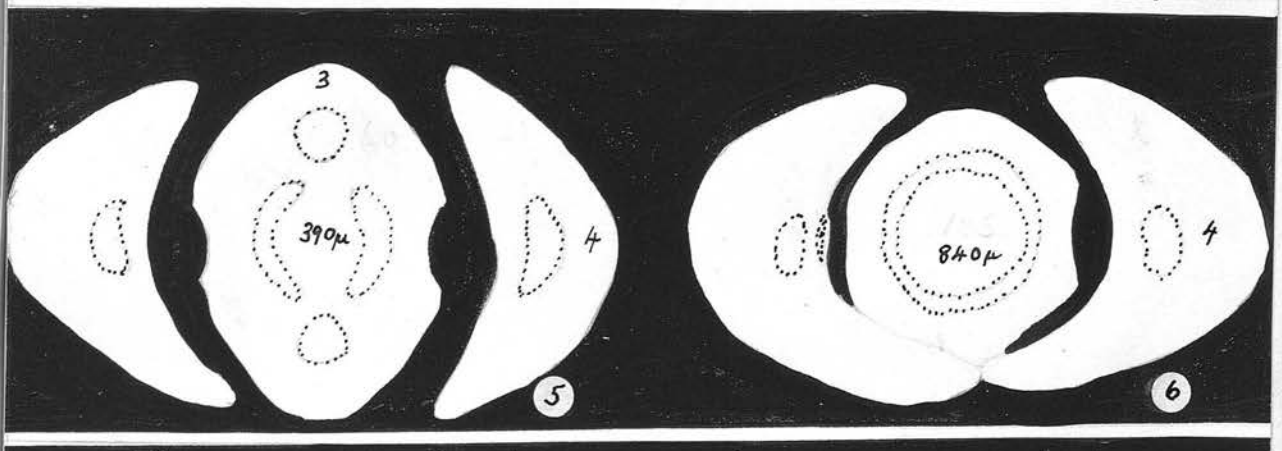


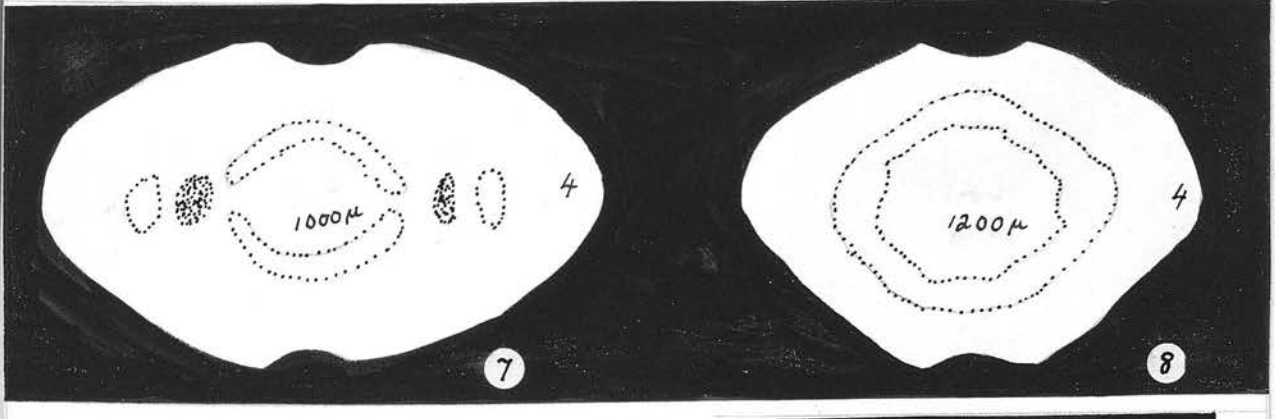
Figure 77. x 500.











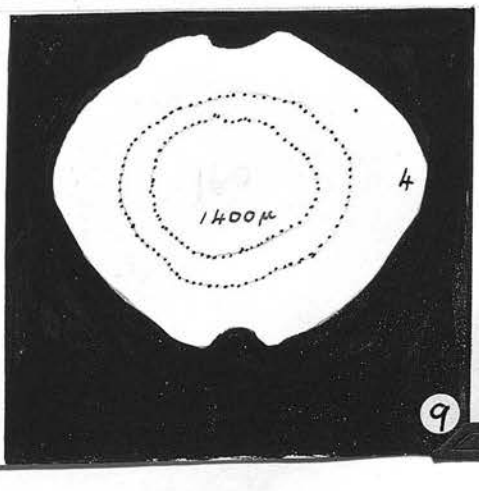


Figure 78.
VINCA.
Bud plan. x 75.

VINCA. x 1000.

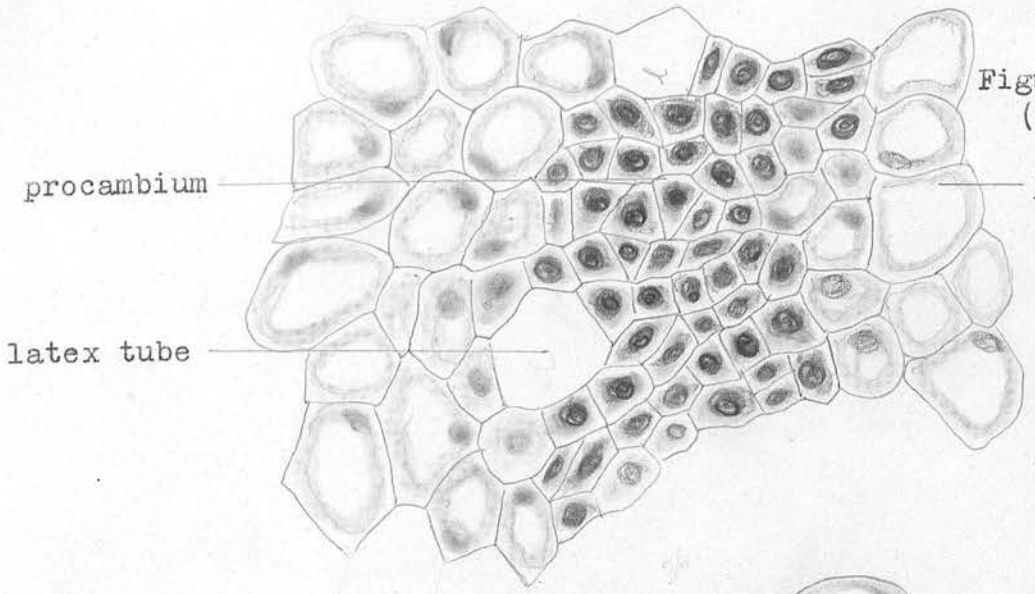


Figure 79.
(70μ)

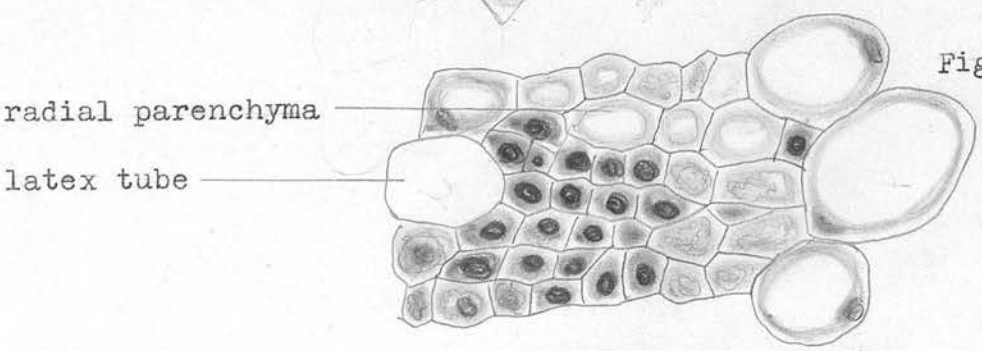


Figure 80.
(140μ)

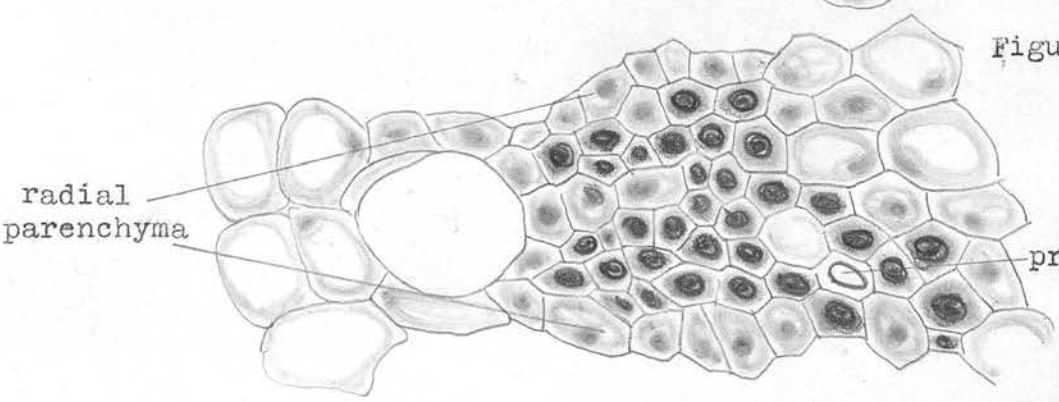


Figure 81.
(190μ)

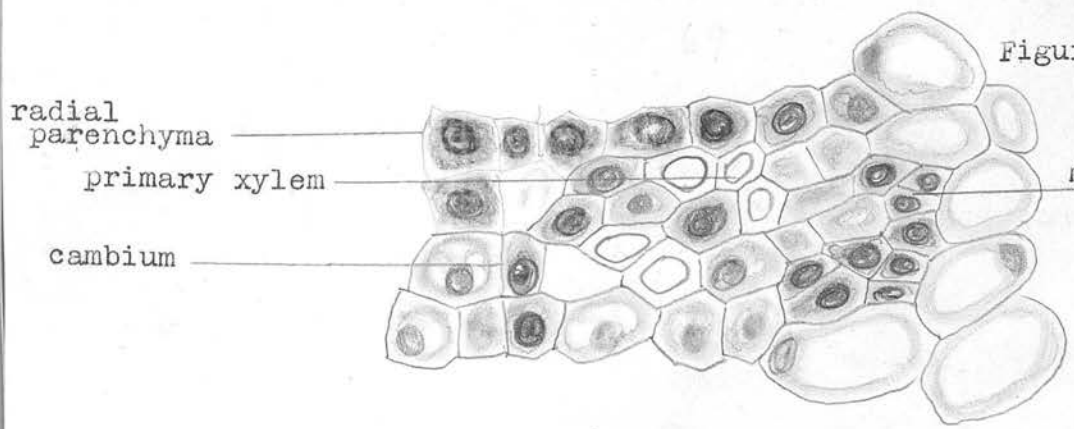


Figure 82.
(450μ)

JASMINUM

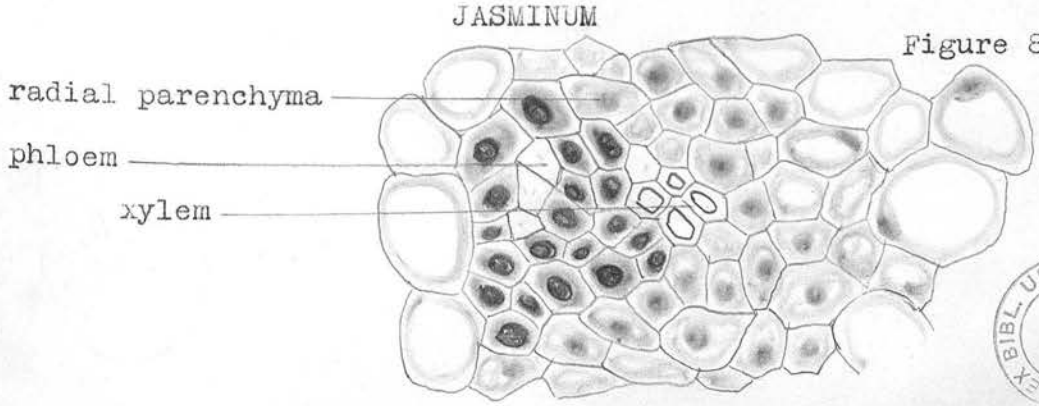
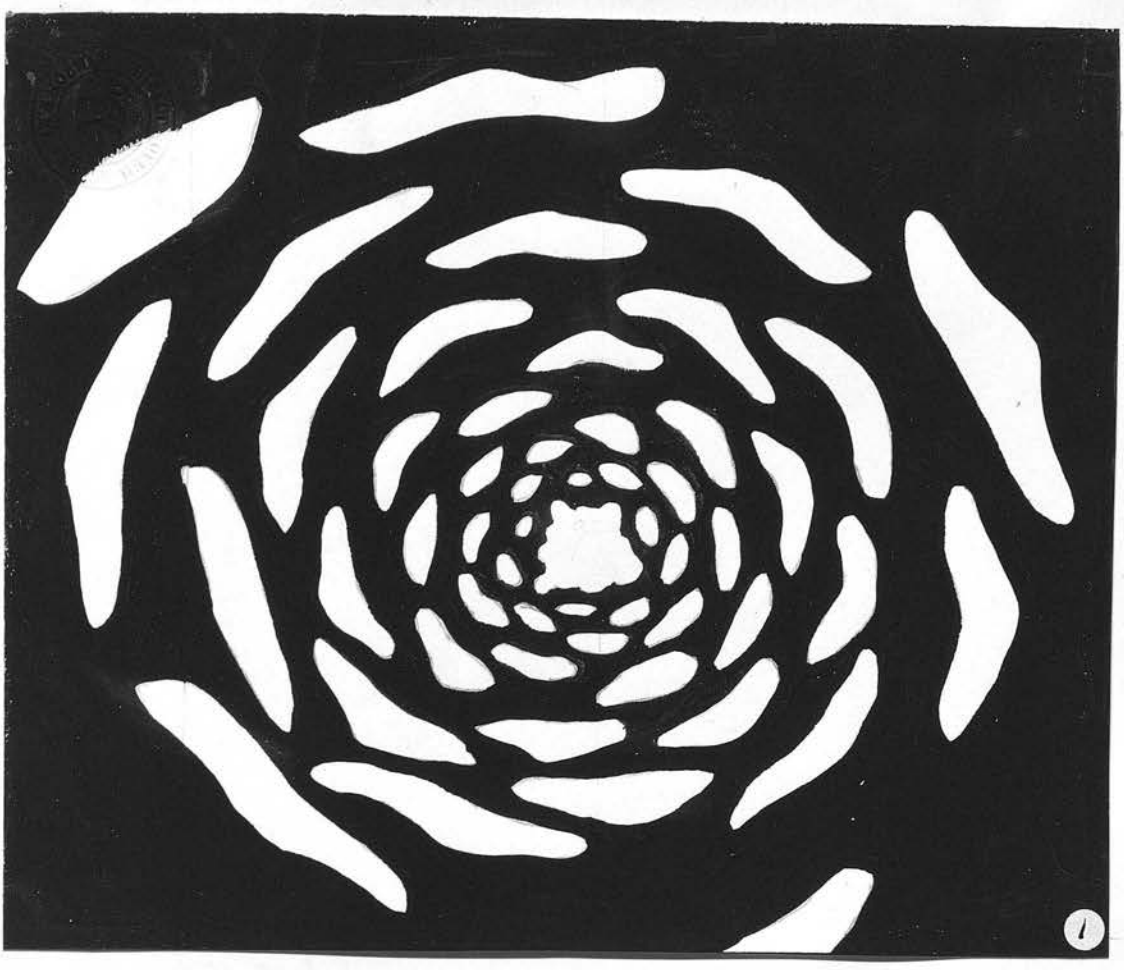
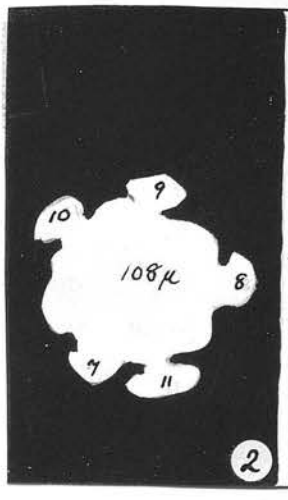
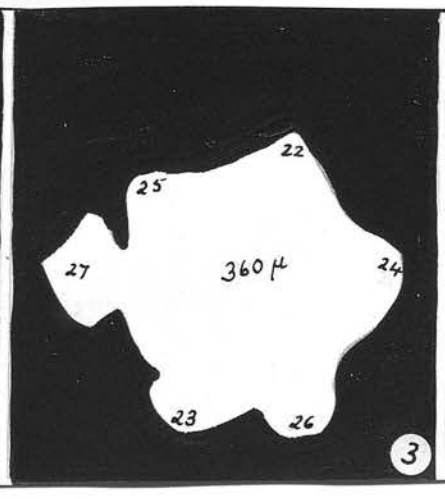


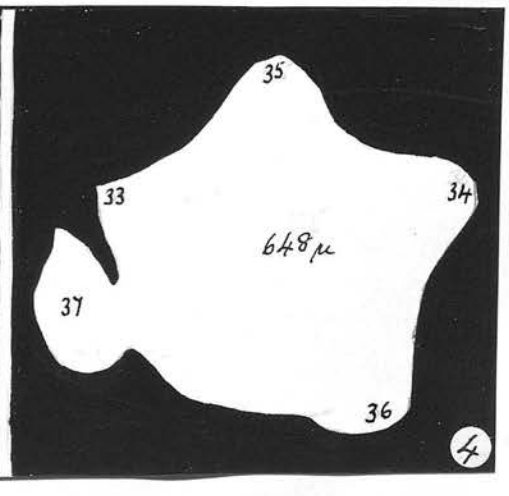
Figure 83.

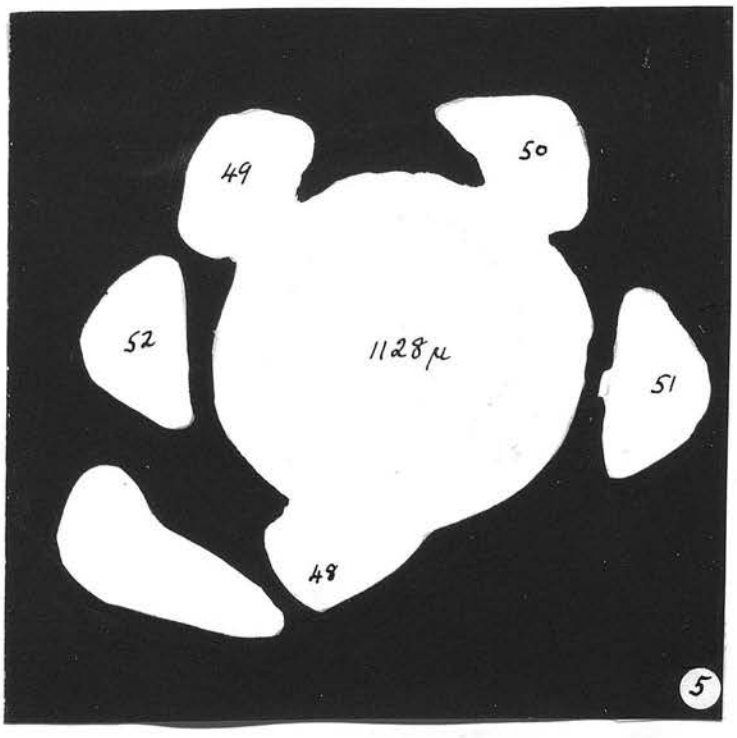
Figure 84. LINARIA. Bud plan. x 75.











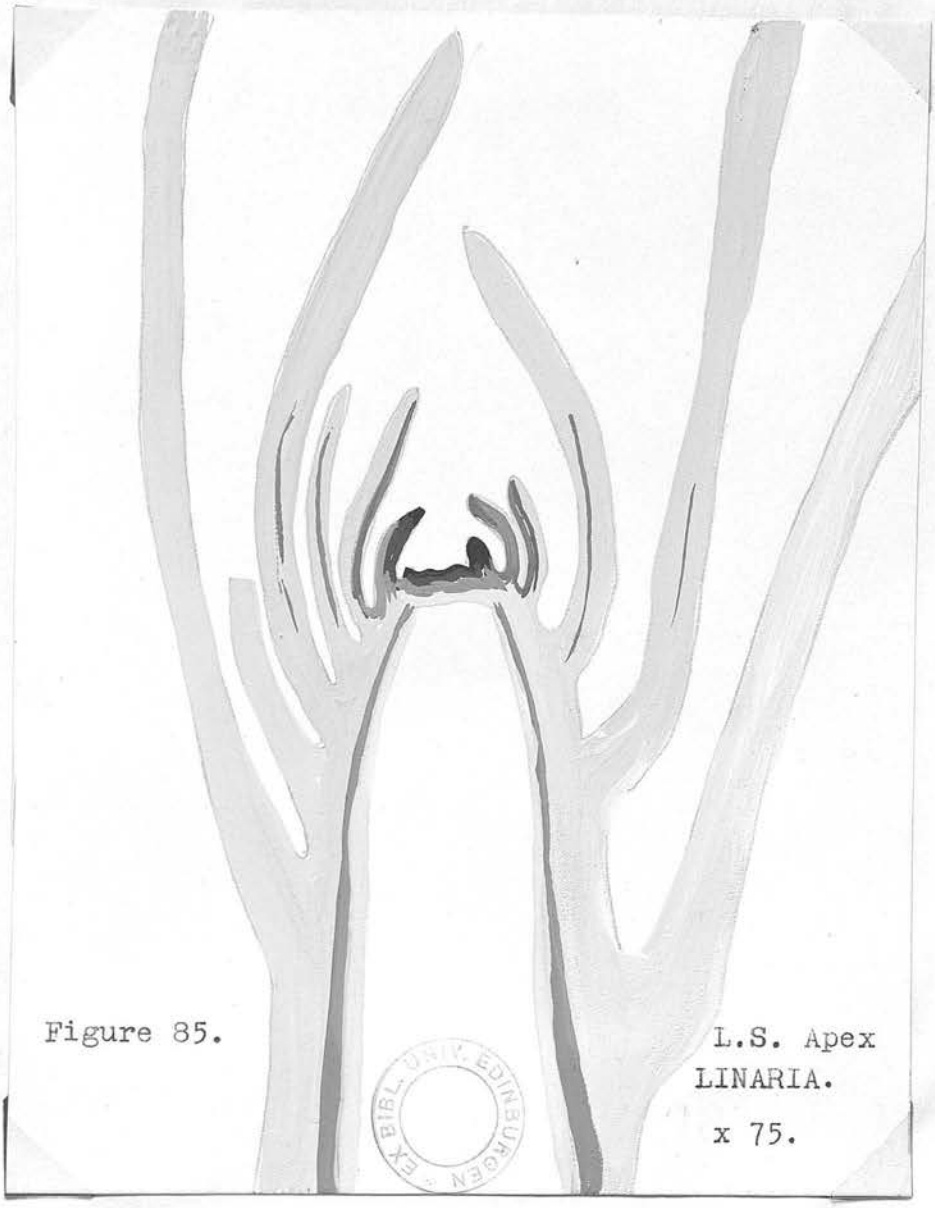


Figure 85.

L.S. Apex
LINARIA.
x 75.

LINARIA. x 1000.

Figure 86 (360μ)

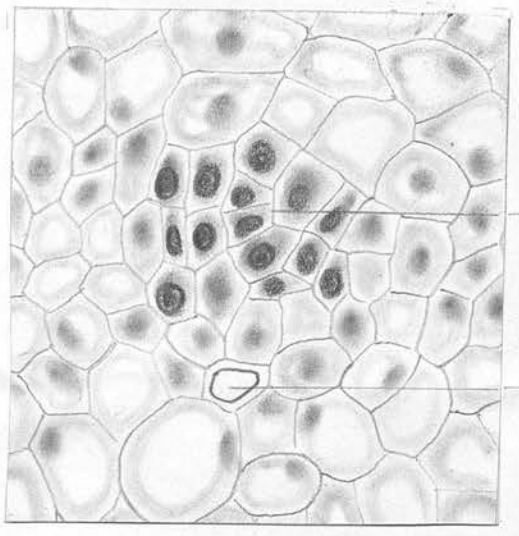


Figure 87. (660μ)

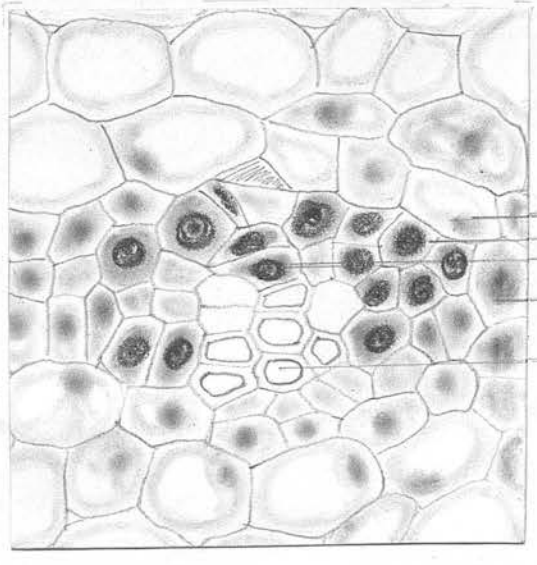
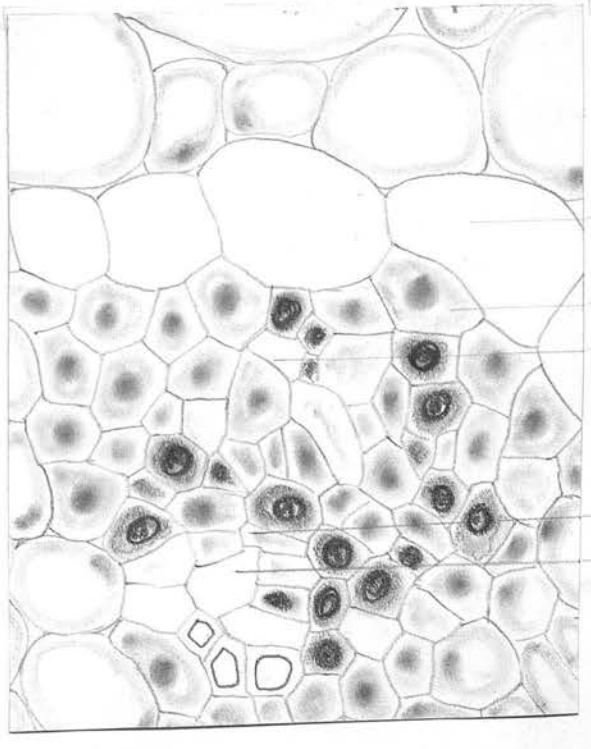


Figure 88. (980μ)



LINARIA. x 1000.

Figure 89. (900μ)

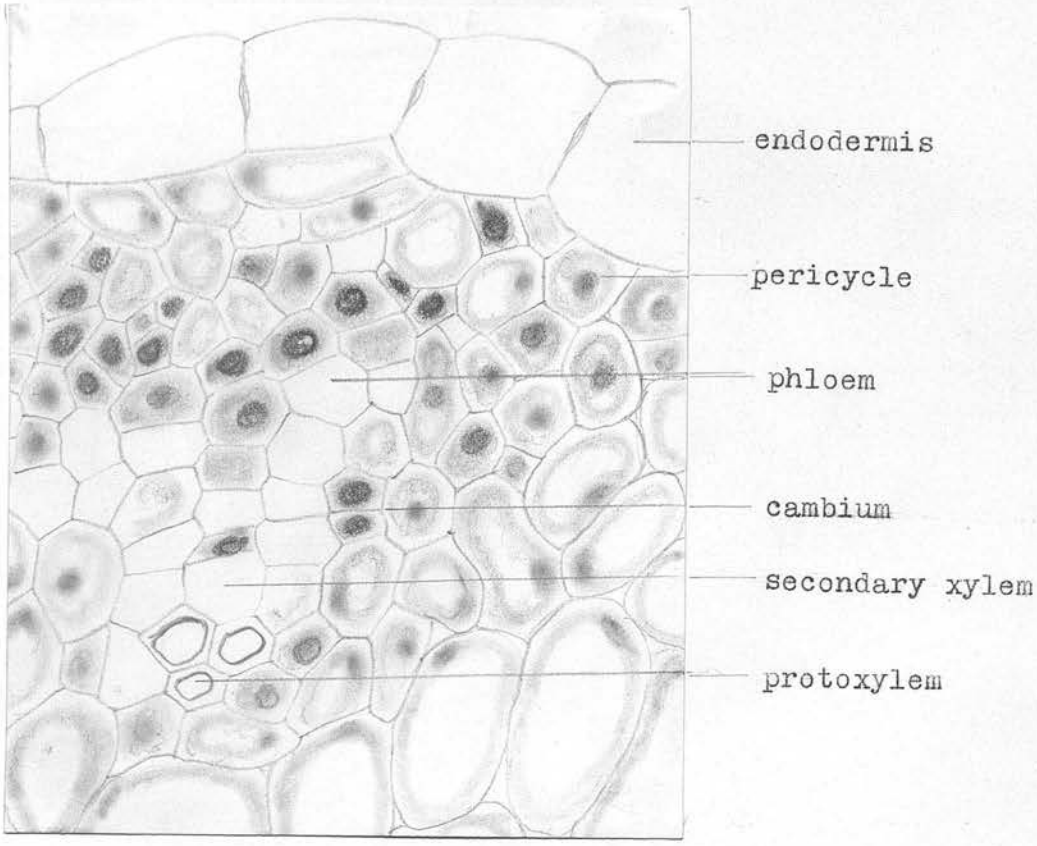
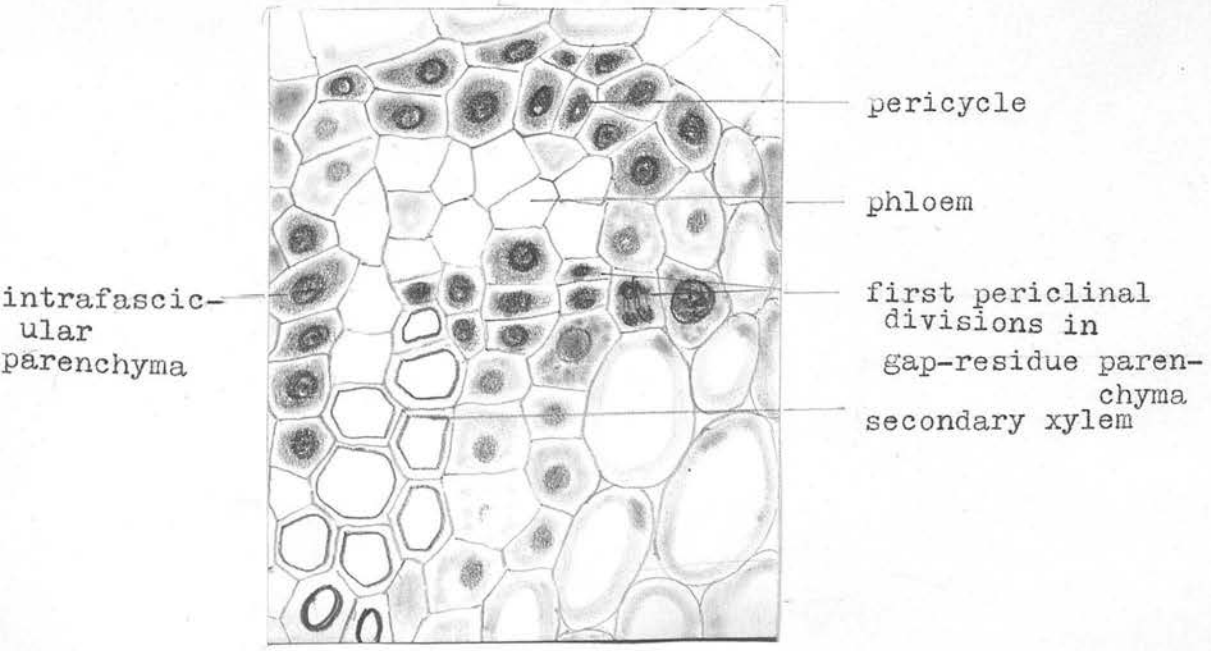


Figure 90. (1500μ)



PLUMBAGO. x 1000.

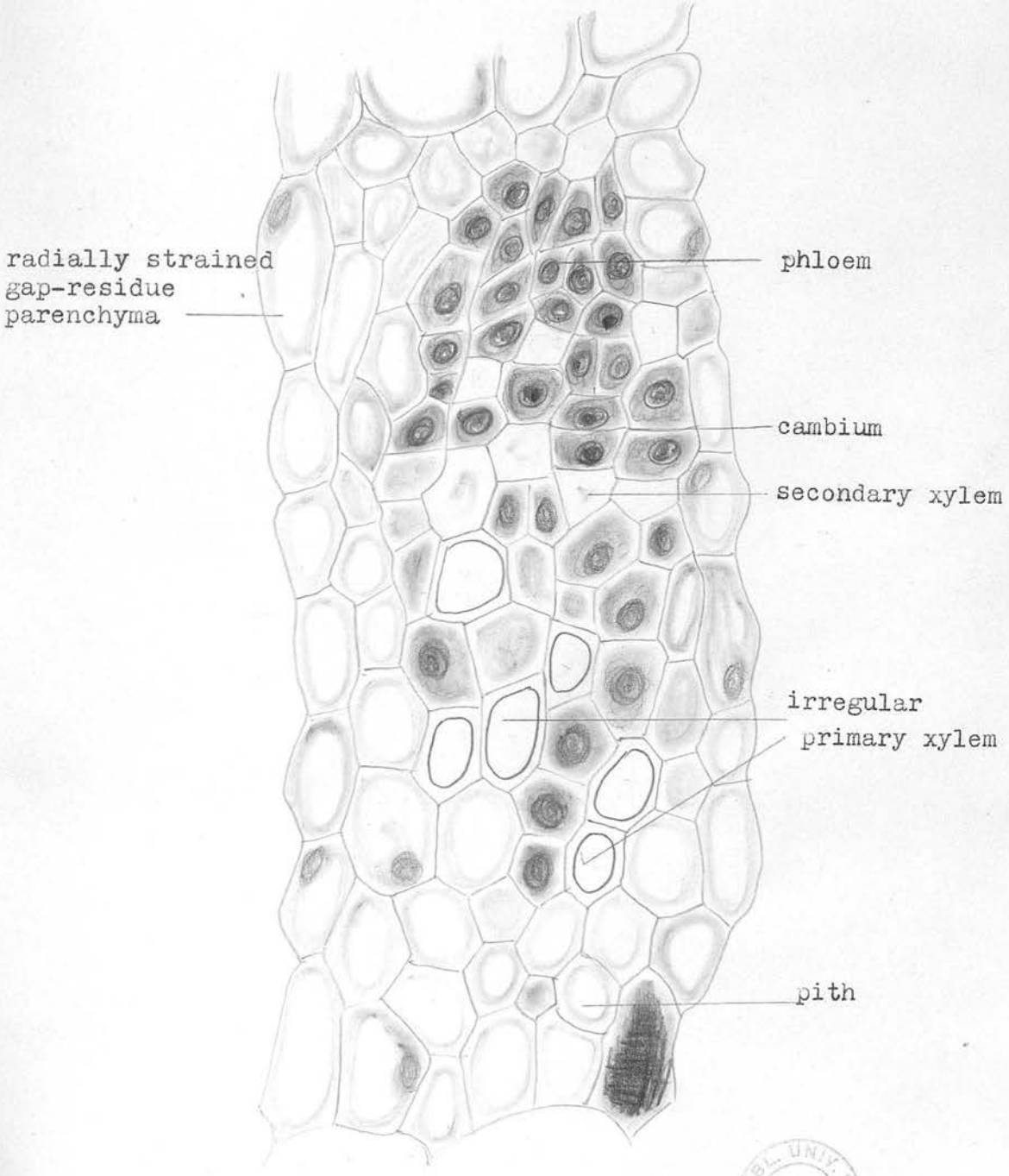


Figure 91.

VERONICA. x 1000.

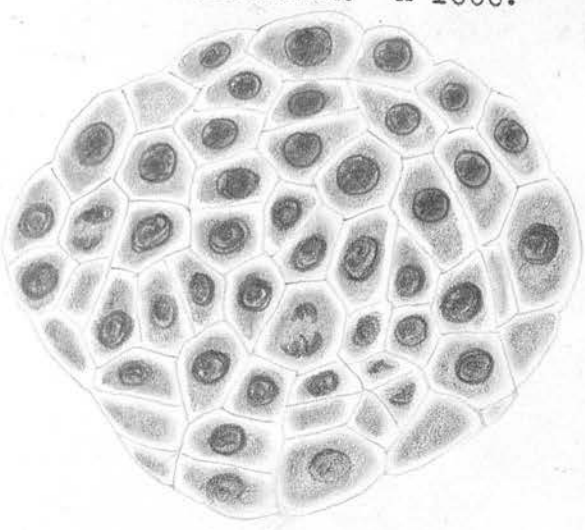
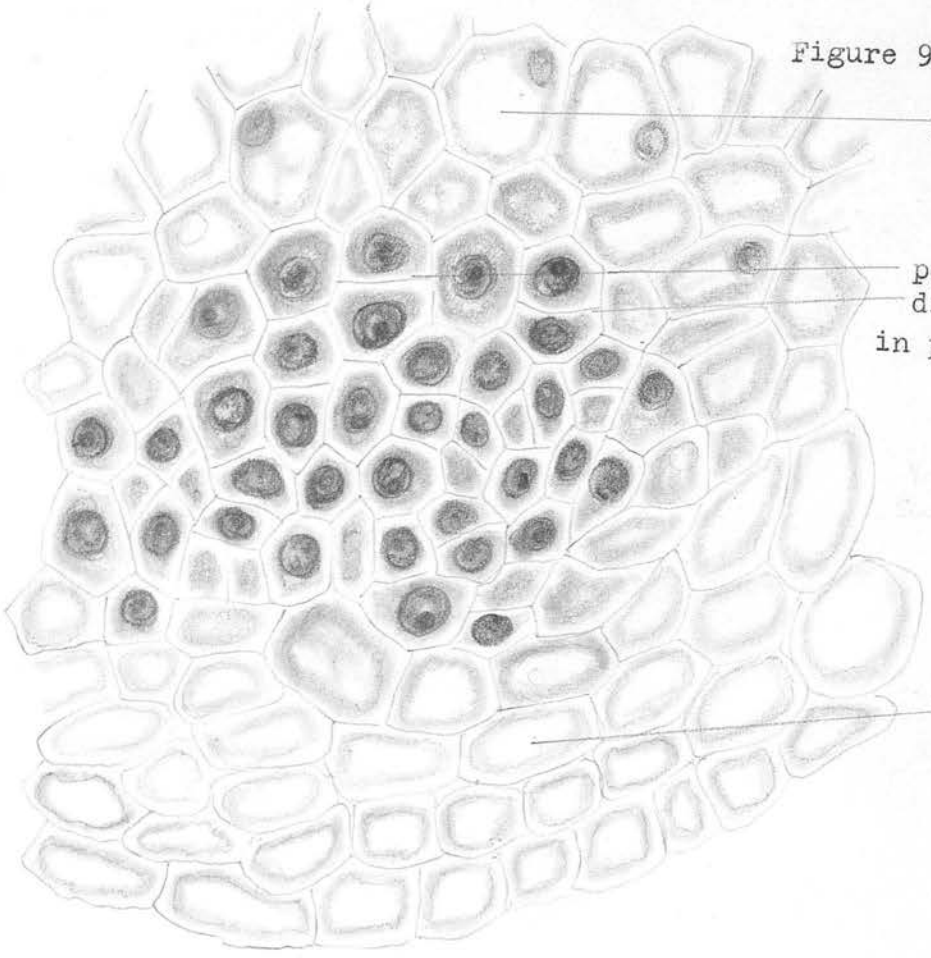


Figure 92.

apical meristem

Figure 93. (140μ)

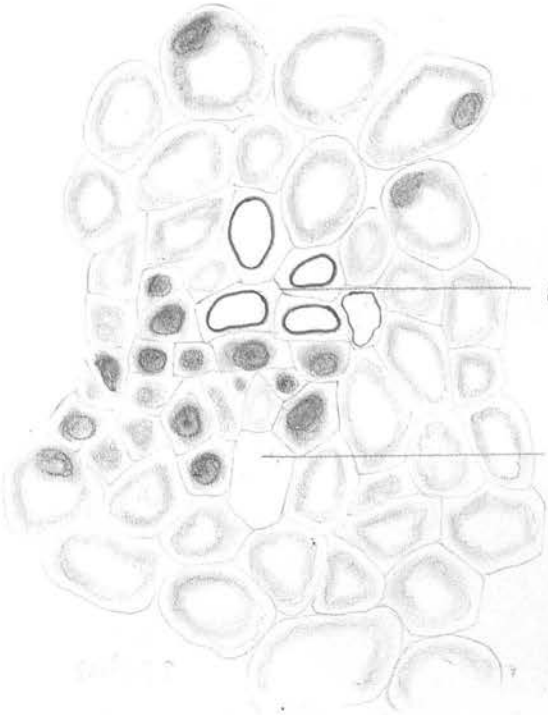


pith

periclinal divisions in procambium

cortex

Figure 94. (370μ)



secondary proto-xylem

primary proto-phloem

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Flower Colours as Natural Indicators.

By Edith Philip Smith, B.A., Ph.D.

(Read 19th June 1930.)

The colours of flowers can be roughly divided into three groups, according to the nature of the pigments involved. Firstly, the pure rose, crimson (and a few scarlet), magenta, and blue series, which owe their colours to anthocyanins (water-soluble sap-pigments). Secondly, the yellow, orange, brown-red series, due to plastid pigments; and lastly the cases where the two types of pigment exist together and modify the final colour. It is proposed to deal with flowers from the first group only.

The use of extracts from coloured flowers, that is, of anthocyanins, as indicators of acidity and alkalinity is an old one; "Syrup of Violets" is mentioned by Robert Boyle (1) in 1664. The tincture developed a bright cherry-red on addition of acid, and a clear green on the addition of alkali. Such crude and unstable indicators as this watery mixture of anthocyanins find no place in the modern battery of sulphone-phthaleins, etc. However, an attempt has been made to study the reaction of the cell-sap of some coloured flowers by using the plant's own pigment as an "indicator," in solutions of known pH value, and comparing these standards with the colour displayed by the cell-sap in the living petal. Buxton and Darbishire (2) made observations on the reaction of the cell-sap of some flowers, but they used watery extracts of dried flowers and titrated them after various periods. When the extracts were treated immediately, little difference was found between the reaction of red (pH 6) and blue (pH 6·5) flowers. After two to three months, extract of blue flowers had a pH of about 7, and the red about pH 5·5. The flowers used include Delphinium, Viola, Lupin, Rose, Peony. The writers suggest that red flowers have a "selective permeability," admitting the hydrion but excluding potassium ion, etc. The blue flowers are assumed to have lost this selective action, hence their more alkaline reaction. Without entering into this

theoretical discussion, it seems to the present writer that the use of living, unmutilated material for comparison avoids death changes and upsetting of the buffer system of the cells. The solutions used for making the colour-standards are sufficiently well buffered to be unaffected by the plant tissue during the extraction of the pigment.

The writer's attention was first called to this interesting possibility by observing the diurnal colour changes of the short-lived flower of the Morning Glory, *Ipomoea Leerii*. These flowers expand from tightly-rolled, magenta-pink buds to fully-opened pure blue trumpets in a very short time (the exact time depending on the temperature at which they are growing). In a few hours the flower begins to fade, changing through violet back to the original magenta, while the corolla crumples inward and rolls up round the mouth of the tube. Obviously this short period of intense activity is accompanied by a change in the reaction of the cells of the corolla. An attempt was first made to determine the pH value corresponding to the colour-stages of the flower, and then to use this in an examination of the effect of external conditions on the daily rhythm.

Method.

In order to determine the reaction of the corolla cells it was necessary to compare them with a standard. The standard series of colours was prepared by extracting the pigment direct into buffer solutions of various pH values, as follows: 5 c.c. of buffer solution were put in a hard-glass test-tube and warmed in a water-bath. The expanded part of a single corolla was cut off and placed in the buffer. Colour began to diffuse out immediately. The tube was quickly brought to boiling-point and kept boiling ten seconds. On cooling, the tissue was seen to be practically colourless. The coloured liquid was decanted to a fresh tube and sealed. The buffers used for this flower were Palitzsch's boric acid M/5–borax M/20 (Clarke (3), p. 88). Comparisons were made with buffer solution which had been boiled for an hour; also by making an extract of the pigment in water and adding in the cold to the buffer. No appreciable difference could be detected between tubes prepared in this way and by direct extraction, so the simpler method was used in all subsequent experiments. It was found that the pre-

pared tubes kept remarkably well if carefully sealed, but there was no occasion to use old standards when fresh ones were so easily prepared. The comparison of the standard colours with the flower-petal was made by means of Pantin's method (8). A portion of corolla was mounted in water on an ordinary micro-slip with a thin cover-glass. By means of the sub-stage condenser, an image of the standard colour-tubes can be focussed in the plane of the object, and by using the mechanical stage it is easy to bring the cells at the edge of the petal alongside of the standard. By using the untouched petal-edge one can actually examine the cells one by one. The most accurate matching was carried out by dispensing with the microscope mirror. The standards were held in a rack standing on a piece of bristol board, the microscope being mounted on a block so as to permit of direct use of the condenser. To avoid the curvature and refracting edges of the image of a test-tube, use was made of the capillary tubes supplied in the B. D. H. Capillator. Three were used for each tint, mounted side by side.

The same method was used with the other flowers examined, and details of the other buffer-mixtures will be found under the corresponding flowers.

Results with Ipomoea Leerii.

The following were the colours observed in the boric acid : borax buffers.

pH.	Colour.
9·24	green
8·2	green-blue
* 7·8	blue * (full day colour)
7·6	blue-violet
7·3	violet
7·09	red-violet
6·7	red-violet
6·0	magenta (bud-colour and faded corolla).

In M/10 HCl the colour was a bright cherry-red.

The plant under observation was growing in the fernery at the Royal Botanic Garden, Edinburgh. The house is kept

at a day temperature of about 21° C. to 26·7° C. The buds open in the early morning, expanding from the tightly-rolled magenta-pink (pH 6·0) to full blue (pH 7·8) in sixty to ninety minutes. If the sun is shining, the flowers may be over by mid-day, while on a dull day they may last till between 4 and 5 p.m. It was decided to examine the effect of light and temperature on the diurnal changes. To do this it was necessary to use cut flowers, but it was found that buds cut either the night before or at dawn and put in water in the fernery opened quite normally and behaved just like buds of the same day left on the vine. There was therefore no objection on those grounds to using cut flowers in the laboratory. For comparison, measurements were taken of a fully expanded flower, cut at 12.30 p.m. on a dull day, temperature 21° C. (colour pure blue).

Diameter of corolla	9·5 cm.
Length of tube to spring of petals	5·1 ,,
Diameter of tube	1·2 ,,
Distance between ribs at circumference	5·5 ,,

Effect of Light on opening of Buds.—If buds due to open the next day (that is, showing a touch of colour between the ribs of the corolla) are cut and placed in darkness at 12 noon, at a temperature of 18·3° C., they will be open by 9 a.m. next day. The following differences from the normal are seen:—size, less than normal; outline of corolla remains pentagonal, never becomes quite circular; the colour remains deep magenta.

Buds exposed to light from a 60-watt electric bulb, 3 p.m. to 9 a.m., temperature 18·3° C., did not open. The ribs of the corolla unfurled slightly, but no more. The colour remained deep magenta, and the flower withered without opening.

Evidence that exposure to light in the bud influences flower-colour is given by the behaviour of a cluster of buds put to open in darkness. Each successive bud to open is redder in colour than the first, and smaller in size.

Effect of Temperature on opening of Buds.—The effect of different temperatures was tried on buds in darkness only. It was found that between 15° to 32° C. buds would open; above 32° C. the flowers withered in the bud. Increase of temperature increases the speed of the opening and withering;

the whole flowering period is over by 9 a.m. at 32 ° C. in darkness. It was found that a temperature of 21 ° C. was the optimum, judging by size of flower and colour of corolla. Even at this temperature the corolla never reached the pure blue of the illuminated flower; a deep violet-blue was the best.

It is evident, therefore, that the full development of the flower is conditioned by both light and temperature, light being the master-factor in determining the reaction of the cell-sap of the corolla and therefore the flower-colour.

The effect of carbon dioxide on the colour-change was also tested. It was found (by enclosing the flower in a large desiccator with soda lime, the desiccator being kept in the greenhouse beside the vine) that the colour-change takes place as usual in CO_2 free air. The colour-change is altered (in the acid direction) in atmosphere with excess CO_2. The flower was put in a small beaker of plain water standing in a desiccator with 500 c.c. of water charged under pressure with CO_2. Cut discs of the corolla are freely permeable to CO_2 over their whole surface, not only at the cut edges. When they are floated on water charged with CO_2 the colour rapidly changes from blue to pink. The CO_2 passes out freely when the discs are removed to plain water, where they quickly resume their original colour. It is therefore suggested that the increased acidity as the flower fades is not due to accumulated respiratory CO_2, but to alterations in the buffer-system of the cells. It has been noted by Irwin (5) that increased respiration in corollas of *Salvia* may be accompanied by decreased acidity, as judged by the colour of the flower.

It is obvious that the metabolic changes accompanying the rapid opening and fading of a flower are exceedingly complicated, and the study of the changing sap-reactions may cast light on some of the angles of the problem.

Primula sinensis.

In turning to the colour varieties of *Primula sinensis* we are dealing with material whose hereditary equipment and genetic behaviour has been extensively studied. Gregory, de Winton, and Bateson (4) have given an account of some of the factors concerned with the production of colour in

the stigma, corolla, calyx, leaves, and stems. These include the following :—

G, giving green stigma ; g, red stigma.
B, blue colour ; b, red.

There is also the factor R, which with B gives magentas.

The flowers studied were " Reading Ruby " (deep magenta, BR), " The Czar " (dark blue, Br), " Etna " (dark red in flowers, stigma, leaves, and stems, bR), and " Light Blue Star." The first observations on these flowers were made in July 1928 at the John Innes Horticultural Institution, on plants from the late Mr. Gregory's strains, carried on by Miss de Winton. The experiments were carried on at University College, Dundee, during the seasons 1929 and 1930, at intervals from March to June. In this case the plants were seedlings from Messrs. Suttons' seeds, in their first year of growth. As the season progressed it was found that the " blue forms " became more purple than during the early months of the year. Whether increasing temperature or light is the cause of this has not yet been ascertained.

The method of extraction of pigment was the same as that used for *Ipomoea*, but because of the small size of the flowers it was necessary to use two corollas to 5 c.c. liquid. In addition to anthocyanins, these flowers contain quantities of flavones, especially in the tube and " eye " of the flower : only the expanded part of the corolla was used for extraction. In order to check the tints assumed by the pigments in different buffers, three sets were prepared :—Palitzsch (3), Clark & Lubs' acid potassium phosphate and sodium hydroxide, [range pH 5·8–8·0] (3), and the British Drug Houses Universal Buffer mixture, pH 2·8–7·2. The borate buffers were not of much use in this case, because the reactions of the flowers proved to lie well on the acid side of neutrality. The colours of the phosphate and the U.B.M. tubes agreed well ; the latter gave a slightly better match with the flower-petal at the acid end of the scale. The corolla of Etna gave a reaction of approximately pH 3·1, of Reading Ruby about 4·0, and of Czar about 4.7.

Comparing the pigments of Etna and Czar, from pH 2·8 to pH 9·24, they appeared to be indistinguishable in tint in solutions of the same pH value. Reading Ruby gave a bluer tint over the significant part of the range.

It is suggested that the pigment of Etna and Czar is the same, and that the factor-difference between them (turning red into blue) is concerned with the reaction of the cell-sap; that is, with the buffer system of the cells. The same pigment in cell-sap of different reactions would show up as a different colour.

The behaviour of Reading Ruby suggests the presence of another anthocyanin along with that occurring in Etna and Czar. Reading Ruby also behaves as a dominant to Etna and Czar.

The pale colour of Light Blue Star made it difficult to prepare standards of a sufficiently deep tint to compare with Etna and Czar, but by diluting pigments from the latter flowers with the appropriate buffers the Light Blue Star could be matched. There again the colour was indistinguishable from those of Etna and Czar. Much flavone was present.

Papaver Rhoeas.

For details of the factorial situation in *Papaver Rhoeas*, see the paper by Newton (6). The plant provides a number of coloured strains, which can be arranged in the following epistatic series: crimson, scarlet, port, claret, mauve. Crimson is heterozygous, and single-factor differences exist between scarlet-port, port-claret, claret-mauve. When tested in buffers the scarlet-port pigments appeared the same. Much flavone is present, especially in black spot at base, which is better removed for test. There again a reaction-regulating function is suggested for a Mendelian factor.

It is natural that in breeding work, especially as methods grow more refined and tests more exacting, attention should be principally focussed on morphological expressions which it is possible to assess with considerable accuracy. The extensive work on inheritance of the more subtle physiological or metabolic characteristics in Maize needs to be extended to many other plants. The work of Small (9) and his co-workers on the hydrion concentration of plant tissues might well be applied most profitably to plants of known genetic constitution and behaviour, and these observations on flower-colour and reaction extended. Many plants exist in which there are full- and pale-coloured, or blue, red and pink varieties; for

example, the Annual Convolvulus (*C. tricolor*), Corn-flower, not to mention Sweet Pea. The greenhouse *Cineraria* is in the act of producing a difficult and interesting problem. The majority of the current florists' strains show blue, violet, magenta, crimson, and rose shades, which might easily be referable to one type of anthocyanin pigment. Recently there have appeared on the market so-called scarlet (really brick-red) strains. The raiser of one of these strains has told the writer that the colour appeared spontaneously in a family of "pink shades." By selection the proportion of brick shades has been increased. The writer is attempting by strict isolation of plants of the brick-red strain to obtain a selfed progeny which will give at least an indication of the diversity of the strain. But the problem remains a chemical one—does the appearance of this new colour indicate a new chemical compound made by the plant (*i.e.* a brick instead of a rose-red anthocyanin): is it a question of sap reaction, or of a development of plastid pigment in addition to sap pigment? If the breeding experiments are successful, it may be possible to attempt an answer to some of these questions, but in the meantime they must be left unanswered.

Summary.

1. The colour of the cell-sap in living cells of the following flowers, *Ipomoea Leerii*, *Primula sinensis*, *Papaver Rhoeas*, was compared with buffer standards prepared with the anthocyanins of the flowers themselves.

2. It was possible in this way to estimate the approximate pH value of the cell-sap corresponding to the colours shown by the flowers under various conditions.

3. *Ipomoea Leerii* has a diurnal colour-range from magenta-pink (bud) to full blue in the freshly-opened flower, corresponding to a pH range of 6–7·8. The development of the full blue is conditioned by both light and temperature. In the dark a violet (pH 7·09–pH 7·3) is the colour attained, between 15°–32° C. At a temperature below 15° C., in light, a similar colour is reached.

4. The commercial colour-varieties "Czar," "Etna," "Reading Ruby," "Light Blue Star" of *Primula sinensis* were examined. The cell-sap of Etna was the most acid (about

pH 3·1), Reading Ruby about 4·0, and Czar about 4·7. The pH value of the Light Blue Star was not determined. The colours obtained from the corollas of Etna and Czar, at the same pH. values in the same buffers, were indistinguishable. It is suggested that the genetic factorial difference between these two flower-colours is one affecting the reaction of the cell-sap of the corolla and possibly of the whole plant.

5. In *Papaver Rhoeas* the two colours "scarlet" and "port" also gave similar tints at the same pH value of the same buffers.

6. The application of this method of investigation to other families where similar colour varieties exist is suggested.

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STOMATAL MOVEMENT AND HYDROGEN ION CONCENTRATION

IN order to test the effect of the hydrogen ion concentration of the cell-sap on stomatal movement, experiments were made with the stomata of *Tulipa*, *Scilla*, *Iris*, and *Tradescantia*. It was found that the hydrogen ion concentration of the cell-sap of the guard-cells was approximately 4·5, while the epidermal cells were slightly less acid. The stripped epidermis was placed in buffer mixtures of known hydrogen ion concentration and left for two, six, twelve, and twenty-four hours respectively. The mixture used was the B.D.H. 'Universal Buffer Mixture'. For each time interval, one set was kept in the light and another in the dark for comparison. It was found that, in the case of *Tulipa*, the stomata were closed between *p*H 1 and *p*H 5, and at *p*H 6, 7, and 9 they were open, the maximum being at *p*H 7. In the dark the stomata were open at *p*H 5, 6, 7, and 9, the maximum being at *p*H 5. In the case of *Scilla*, the closure continued up to *p*H 6 in light, to *p*H 5 in the dark, with a maximum at *p*H 7.

In order to avoid the possibly toxic effects of the buffer mixture, the experiments were repeated with solutions of carbon dioxide, of *p*H values 4·4, 4·6, 4·8, and 5·0. In all these, the stomata of *Tulipa* were open, the greatest opening being at 4·4 in light and 5·0 in dark. *Scilla* showed closure at *p*H 4·4 and 5·0 in light and dark, opening at 4·6 and 4·8. *Iris* stomata were open throughout the whole range in light, and closed at 4·8 and 5·0 in dark, with a maximum opening at 4·4. *Tradescantia* showed a maximum opening at 4·4 in light, and closed at 4·4 in dark, with greatest opening at 5·0.

It is evident from this that the stomata of these forms are susceptible to changes in the *p*H value of the sap produced by carbon dioxide, and that the guard-cells respond by movement to these artificially produced changes. The experiments of which this is a preliminary account are still in progress, and it is intended to extend the observations to dicotyledons as well as to monocotyledons.

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M. S. JOLLY.

University College, Dundee,
March 3.

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EDITH PHILIP SMITH, B. A., Ph. D.

THE CALIBRATION OF FLOWER COLOUR INDICATORS

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PROTOPLASMA

INTERNATIONALE ZEITSCHRIFT FÜR PHYSIKALISCHE CHEMIE DES PROTOPLASTEN
INTERNATIONAL JOURNAL OF THE PHYSICAL CHEMISTRY OF PROTOPLASM
ARCHIVES INTERNATIONALES DE CHIMIE PHYSIQUE DU PROTOPLASMA
ARCHIVIO INTERNAZIONALE DI CHIMICA FISICA DEL PROTOPLASMA

UNTER BESONDERER MITWIRKUNG VON
ROBERT CHAMBERS (NEW YORK) UND **WILLIAM SEIFRIZ** (PHILADELPHIA)

HERAUSGEGEBEN VON
JOSEF SPEK (HEIDELBERG) UND **FRIEDL WEBER** (GRAZ)

Alle biologischen Disziplinen mit kausaler Fragestellung sind an der physikalischen Chemie des Protoplasmas interessiert. Um das harmonische Zusammenarbeiten der verschiedenen Wissenszweige, um den Überblick und die Synthese auf dem Gebiete der Protoplasmaforschung zu ermöglichen, bedarf es eines eigenen die Ergebnisse zentralisierenden Organes.

PROTOPLASMA, die Internationale Zeitschrift für Physikalische Chemie des Protoplasten ist schon in den ersten Jahren des Erscheinens zu diesem von Vielen längst ersehnten räumlich-geistigen Bande aller Protoplasma-Forscher geworden.

Die zunächst liegende Aufgabe der Internationalen Protoplasma-Zeitschrift muß es sein, die Schwierigkeiten des geistigen Kontaktes abzubauen, unter denen jeder einzelne Forscher und die gesamte Wissenschaft auf diesem Gebiete infolge der Heterogenität der beteiligten Disziplinen leiden. Die Physiologie der Pflanzen und Tiere, die allgemeine Cytologie, die verschiedenen medizinischen Wissenszweige (wie Physiologie, Pharmakologie, Pathologie, Histologie), die alle in der physikochemischen Erforschung des Protoplasmas die Lösung so mancher Rätsel erhoffen, haben den dringend gewordenen Austausch an Gedanken, Methoden, Erfahrungen bisher nur allzu spärlich und zögernd in die Wege geleitet.

Mit der Beseitigung der trennenden Schranken wird sich von selbst die Erreichung des wichtigsten Zieles der Internationalen Protoplasma-Zeitschrift ergeben: Durch die gegenseitige Anregung der einzelnen Disziplinen neue Blickpunkte zu gewinnen, neue Arbeitsmöglichkeiten zu schaffen.

Die Protoplasmaforschung muß geformt, geprägt, organisiert werden. Das kann heute nicht mehr ein Einzelner, sondern nur eine Arbeitsgemeinschaft, an der alle kausal-biologischen Disziplinen sowie die physikalische Chemie beteiligt sind.

Das Arbeitsgebiet der neuen Zeitschrift bedarf der Abgrenzung mit möglichster Schärfe. Die eigensten Gebiete der Protoplasmaforschung, die zu pflegen die neue Zeitschrift sich in erster Linie zur Aufgabe macht, seien besonders namhaft gemacht:

Kolloidchemie des Protoplasten. Physiko-chemische Eigenschaften des Protoplasten (wie Oberflächenspannung. Viskosität. Quellung. Elastizität. Adhäsion. Adsorption. pH. rH. Et cetera). Mikrochemie des Protoplasten im kausal-analytischen Sinne. Elektrometrie des Protoplasten. Vitale Protoplasmastruktur. Osmotische Zustandsgrößen des Protoplasten. Permeabilität. Plasmolyse. Narkose. Cytolyse. Hämolyse. Vitalfärbung. Physiko-chemische Grundlagen der Protoplasmabewegung. Microdissection. Ultra- und Polarisationsmikroskopie in Anwendung auf die Protoplasmaforschung. Mechanismus der Zell- und Kernteilung. Protoplasma-Aktivierung. Physiko-chemische Grundlagen der pharmakologischen und Gift-Wirkungen, der Resistenz und Empfindlichkeit sowie der Strahlenwirkung auf den Protoplasten. Pathologie des Protoplasten (Physikalische Chemie des Tumorprotoplasten). Modellversuche an leblosen Kolloiden, insofern sie zur Klärung der Protoplasteneigenschaften beitragen.

Die Zeitschrift erscheint in zwanglosen Heften, von denen 4—5 einen Band von 40 Druckbogen bilden. Die Abhandlungen, Kleineren Mitteilungen, Sammelreferate und kritischen Referate können in deutscher, englischer, französischer oder italienischer Sprache verfaßt sein.

Subskribenten werden die einzelnen Hefte zu einem Vorzugspreis geliefert, der nach Erscheinen des Schlußheftes eines Bandes erlischt. Der Preis des ganzen Bandes erfährt somit für Nicht-Subskribenten eine Erhöhung.

THE CALIBRATION OF FLOWER COLOUR INDICATORS

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With Plates V and VI

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INTRODUCTION

It has long been known that the anthocyanin pigments of plants are capable of acting as indicators of hydrogen ion concentration. The use of extracts of coloured flowers for this purpose dates back to Boyle, 1664 (2), who employed syrup of violets. More recently such natural indicators as blueberry juice and red cabbage have been used by Watson, 1913 (28), and Walbum, 1913 (27) and McClendon, 1914 (15). Since in the majority of cases the anthocyanins are in solution in the cell-sap, they may be used to determine the reaction of the cell-sap of suitably coloured cells. This was first done by Schwarz, 1892 (22). He correlated the change from red to blue during the anthesis of flowers of *Pulmonaria*, *Anchusa* and *Lathyrus* with a decrease of acidity. Later, Willstätter, 1914 (29) moted that the same anthocyanin pigment was responsible for the colour of the rose and the cornflower, and gave a value of pH 5·5 for the rose petal and pH 7·2 for the cornflower. He also found that in buffered solutions the rose goes blue at pH 7·2, and the cornflower red at pH 5·5. Haas, 1916 (11) investigated the anthocyanin pigments of a number of plants. These natural indicators have also been used by Crozier, 1919 (7); Irwin, 1919 (12); Atkins, 1922 (1); Smith, 1923, 1929, 1931 (26, 27, 28); Brooks, 1926 (3); Buxton and Darbishire, 1929 (4), etc.

METHOD AND MATERIAL

Before attempting to evaluate the reaction of cell-sap in terms of a natural indicator, it is necessary to calibrate the indicator. This was done as described below.

The pigments were extracted directly, by heating the flower-petals in small portions of buffer mixtures. The petals were dropped, without

* Part of the cost of illustration was borne by grants from the University Court of the University of St. Andrews and from the Carnegie Trust.

preliminary crushing, into the buffer mixture in a Pyrex test-tube, heated to boiling point over a small bunsen flame, boiled for five seconds and allowed to cool. As the tube cooled the petals were gently pressed with a glass rod to assist extraction. Owing to the very varying amounts of pigment present in different flowers, the volume of buffer solution and the quantity of petals needed to give an extract of suitable strength of colour had to be found by trial for each flower. Only the coloured portions of the flowers were used, and in *Primula*, where there is a large localized production of flavones round the eye, that part of the flower was discarded.

Since it is well known that anthocyanins change their colour somewhat rapidly in watery solutions, the time of cooling and extraction was standardized at twenty minutes. After that time the extract was poured off into a small Pyrex tube, sealed with a cellophane-wrapped rubber stopper and then paraffined. Treated in this way the extracts kept their colour remarkably well, especially in the more acid range. Those at the more alkaline end of the range —pH 8 to 11— did not keep so well.

For comparison, samples of the extracts were sealed off in hardglass capillary tubes (those supplied with the British Drug Houses 'Capillator' outfit). This is a very useful way of keeping a number of standards in compact form, but the small depth of solution makes the tints too pale for direct comparison with the flower-petal.

The buffer solutions were tested independently, and it was found that the short period of heating used did not alter the pH.

The following buffer mixtures were used:

1. British Drug Houses 'Universal Buffer Mixture'. [See Prideaux and Ward (19)].
2. Sørensen's Primary and Secondary Phosphates. [See Clark, p. 114 (5)].
3. Palitzsch's Boric Acid-Borax mixture. (Clark, p. 115).

The Universal Buffer Mixture covers a pH range of 2·7—11·4: the phosphate mixture covers pH 5·288—8·043: the borate mixture from pH 6·77—9·24. It was found that there was no difference in the tints given by any one pigment over the same pH range with the three buffers, so that the Universal Buffer Mixture was standardised for convenience.

For comparison a watery extract was made of the pigments of the red, magenta and blue *Primula sinensis*, and of *Ipomoea Leerii*. The extract was evaporated to dryness, the residue taken up in alcohol, and added to the buffer in the cold. The results were not nearly so satisfactory: the tints obtained were weak by comparison with the direct extracts.

The method used by BUXTON and DARBISHIRE (4) was also tried. This consists of crushing the petals with pumice powder and adding the crushed mass to buffer solutions. The colours are not so clear as the direct extracts, and quickly fade, as stated by the authors. This is probably due partly to adsorption of the pigment by the pumice powder. When immediate comparison was made between the extracts prepared in this way and the direct extracts, it was seen that, for the same flower and the same pH value, the colours were quite comparable, so that the simpler method was used for all further experiments.

Although repeated comparisons between freshly made extracts and extracts kept for varying lengths of time convinced the writer that a few days or even a week made no difference to the colours in carefully sealed tubes, nevertheless to be on the safe side the colours were recorded as soon as possible after decanting from the residue and always within four hours of making the extract. The writer is inclined to think that the reports of rapid fading of anthocyanin extracts are in part at least due to the use of unbuffered solutions.

Matching the colour standards with the living petal was carried out by PANTIN'S method (17). A shallow cell was made by spinning a ring of cellulose paint on a thin microscope slide. In this a piece of petal with as much intact edge as possible was mounted in water, and sealed in with a thin cover-glass vaselined to the ring. The microscope was mounted on a block so as to allow of the direct use of the condenser without a mirror, and the colour-standards, held in a test-tube rack, were focussed by the condenser in the plane of the image. A sheet of bristol board placed on the bench behind the test-tubes acts as a reflector, and the matching was done by daylight (north light). In this way the coloured cells of the petal and the tubes of standard colour were viewed side by side by transmitted light. (The distance between the test-tubes should be such that only one is in view in the microscope field at one time.) By using the mechanical stage the petal edge can be brought alongside the image of the colour tube. When a correct match has been found, the tube appears like an extension of the petal [cf. COCKING, 1926 (6)]. The colour range of each flower was recorded with matt watercolour (poster paints) on bristol board. The difficulties of recording in matt paint the virage of a coloured liquid led the writer to experiment with artificial standards, made up with known stains and indicators, whereby the colour range of a pigment could be reproduced if necessary. The results with *Delphinium tatsienense* are given as an indication. At pH 1, the flower pigment

is red-violet: this is matched by Brom-cresol Purple at pH 7. At pH 3, the pigment is violet: matched by Brom-cresol Purple at pH 8. At pH 6, the pigment is blue-violet: matched by Brom-cresol Purple with a trace of Brom-thymol Blue at pH 8·7. At pH 7, the pigment is grey-blue: matched by Brom-phenol Blue at pH 4·4. The pigment is pure blue at pH 7·3: matched by Brom-thymol Blue with a trace of Brom-cresol Purple at pH 8·7: this exactly matches the living petal.

RESULTS

The following flowers were examined and the petal-pH estimated.

NAME	COLOUR	pH OF PETAL
Group I. 'Reds'		
Anemone "St. Brigid"	scarlet	3·2
Cineraria, Feltham Beauty strain (Sutton)	brick-red	6·2?
„ *grandiflora* strain (Storrie)	brick-red	6·2?
Hibiscus sp.	scarlet	3·1
Nicotiana, "Sander's hybrid"	very dark red	—
Primula beesiana × *bulleyana*	bright pink	about 4
„ *bulleyana*	orange yellow	—
„ *cockburniana*	orange	—
Tulip "Cramoisie Brilliant"	scarlet	—
Primula japonica, "Near Briscoei"	salmon pink	3·1
Group II. 'Magentas'		
Anemone "St. Brigid"	puce	4·5
Cineraria, Feltham Beauty (Sutton)	deep magenta	6·4
Petunia, hybrid	rose pink	5·0
Primula burmanica	pale lilac	4·5
„ *japonica* (type)	magenta	3·5
„ *pulverulenta*	magenta	3·1
„ *sinensis*, 'Etna'	deep crimson	3·1
„ „ , 'Reading Ruby'	deep magenta	3·4
„ „ , 'Czar'	violet blue	5·2
Group III. 'Blues'		
Anemone "St. Brigid"	purple	4·5
Cineraria, Feltham Beauty (Sutton)	royal blue	—
„ "Wonder Queen" (Storrie)	pure blue	7·4
Delphinium tatsienense	pure blue	7·3
Ipomoea Leerii	pure blue	7·8
Petunia "Poors's Blue"	royal blue	5·0
Viola cornuta	violet	4·5
„ *odorata*	violet	4·4

TABLE I.

FLOWER	pH OF BUFFER		
	1·2	3·2	4·4
'Reds'			
Anemone	scarlet	brick	crimson
Cineraria	orange red	scarlet	red
Hibiscus	scarlet	cherry	crimson
Nicotiana	scarlet	brt. crimson	brown purple
Primula beesiana × *bulleyana*	brt. cherry	cherry	—
Primula bulleyana	salmon	pale pink	pale pink
Primula cockburniana	salmon	—	—
Primula japonica	orange pink	crimson	red violet
Tulip	orange pink	salmon	—
'Magentas'			
Anemone	cherry	magenta	red violet
Cineraria	scarlet	crimson	magenta
Petunia	—	pink	—
Primula burmanica	magenta	red violet	red violet
Primula japonica	cherry	magenta	purple
Primula pulverulenta	cherry	crimson	crimson
Primula sinensis 'Etna'	crimson	magenta	red violet
Primula sinensis 'Ruby'	crimson	magenta	purple
Primula sinensis 'Czar'	cherry magenta	magenta	red violet
'Blues'			
Anemone	cherry	magenta	violet
Cineraria	cherry	purple	violet red
Cineraria 'Wonder Queen'	crimson	red violet	blue violet
Delphinium	red violet	violet red	violet
Ipomoea	cherry	cherry	crimson
Petunia	—	magenta	—
Viola	cherry	magenta	violet

It was found that, as stated by Buxton and Darbishire (4), the anthocyanin flower pigments could be placed in three groups: red, magenta (intermediate) and blue. Those belonging to the red group passed from bright scarlet-red at the acid end of the scale through brown-red, purple, usually to brown-red again at the alkaline end. The magentas ranged from bright cherry-red through shades of magenta and purple to

Colour Chart

SOLUTIONS

5·2	6·2	7·4	7·8	8·7	9·6
red grey	grey	olive brown	—	yellow green	yellow green
brick	brown	brown pink	—	brown pink	brown pink
crimson	rose crimson	burgundy	—	brown red	brown red
red violet	red violet	burgundy	—	brown red	brown red
salmon	—	brown pink	—	—	olive
brown pink	brown pink	flesh	—	brown	brown
pink	—	brown pink	—	—	brown
brick	red grey	grey	—	brown	—
pale pink	grey pink	brown pink	—	brown red	brown red
violet	grey violet	grey blue	—	grey	green
red violet	red violet	purple	—	violet	violet
magenta	—	red violet	—	—	peacock
puce	grey violet	grey	—	olive	green
red violet	violet	grey violet	—	grey green	olive green
red violet	puce	grey purple	—	green	—
violet	brown purple	red grey	—	grey green	green
red violet	violet	puce	—	grey	grey green
puce	grey violet	grey	—	grey green	olive green
blue violet	blue	cobalt	indigo	blue green	olive green
violet	violet	violet blue	pure blue	blue grey	olive green
blue violet	blue	blue	blue	indigo	indigo
violet	violet	pure blue	blue green	peacock	green
crimson	red violet	violet	blue	blue green	blue green
violet	—	—	—	peacock blue	peacock green
ultramarine	cobalt	blue green	grey green	olive green	yellow green

grey and yellow-green (the tint depending upon the amount of flavone present): they never showed a true blue tint at any pH value. The blues were cherry-red in acid solution, pased through violet to blue and green at the alkaline end, the green again being due to the presence of flavones which develop a strong yellow colour in alkaline solution. This particular colour-change has long been considered as

definitive of anthocyanins: it is now obvious that it is only characteristic of *some* anthocyanins.

It is not always possible to place a flower in its appropriate group merely by inspection: see for example *Viola*, *Petunia* "Poor's Blue", *Primula sinensis* "Etna" and the purple *Anemone*. Again, it was found in three cases-the royal blue and deep magenta Cinerarias, and the very dark red hybrid *Nicotiana*—the pigment was so concentrated in the cell-sap that it was not 'indicating'.

The colour-ranges of these flower-pigments are given in Table I, and a selection shown in Plates V and VI.

DISCUSSION OF RESULTS

This investigation was primarily undertaken in an effort to gain further information about the hydrogen ion concentration of cells by a method which did not involve either sectioning or the introduction of an indicator into the cell. The Range Indicator Method of Small (23) and his co-workers has yielded and is yielding results of great value to our knowledge of the pH of plant cells and the buffer systens involved, but it is laborious in application and cannot be applied without destroying the tissue examined. This obviously invalidates it for application to such problems as the metabolic changes accompahying the opening and withering of flowers etc. This has been referred to above [Schwartz (22)]. An account of the process in *Ipomoea Leerii* has been published by the writer [Smith (25)], in which the effects of light, temperature and carbon dioxide concentration on the flower-colour are considered.

In order to use the flower's own pigment as an indicator, it is first necessary, as stated above, to determine the colour-range of the pigment in solutions of known pH value. The question then arises, how far are we justified in equating the tints obtained *in vitro* with those of the living cell? The chief difficulties seem to be these: i) Is the pigment or mixture of pigments altered by the method of extraction? ii) Is the colour-range affected by the nature of the buffer mixture? iii) Does the presence of other substances in the crude extract alter the colours?

It is possible that boiling with the more acid buffers might hydrolize the anthocyanin to the sugar-free compound, but it is unlikely that a serious amount of change could take place in the brief time of heating used in these experiments. Then again, it is unlikely that even the most alkaline buffers used (pH 8·6 and 9·7) would decompose the pigments

to any great extent, since 15 % NaOH is described as "very dilute alkali" by KARRER and WIDMER (13) in their investigations.

It has been shown [SMALL (23)], that the principal buffering agents in plant juices are phosphoric, citric, malic and oxalic acids and their salts, together with the carbonic acid-bicarbonate system and to a lesser extent amino-acids such as asparagin and proteins (e.g tuberin in potato). Of the buffer mixtures used, the SØRENSEN mixed phosphates was the most closely comparable to the natural plant buffers. It was found, however, that there was no difference between the colours obtained with the phosphate mixture and the B.D.H. Universal Buffer Mixture, which contains phosphoric, phenyl-acetic and boric acids. These artificial mixtures are much more strongly buffered than natural sap, so that the probable addition of sap acids and a trace of protein in the extraction will not appreciably shift the pH of the extract. The fact that, except in the most alkaline solutions, the direct extracts do not become cloudy or precipitate for a considerable time shows that any protein extracted with the pigment remains in solution and does not remove pigment by adsorption.

If it were found impossible to duplicate the petal-colour in *any* artificial buffer mixture, one would be obliged to reconsider the whole question: but in every case in the flowers belonging to the magenta and blue groups an apparently perfect match has been possible with one or more buffer mixtures.

It seems therefor quite legitimate to consider that the anthocyanins show colours in buffer solutions which are comparable to those shown in the living cell, and that they may therefor be used for estimating the pH value of the cell-sap.

While it is easy to match the colours of the blue and magenta groups, in the red group there are two zones of red-purple or brown-purple; one on the acid and one the alkaline side of neutrality; so that in some cases it was not possible to decide which of two apparently matching tints represented the real pH of the petal.

A consideration of the pH values estimated for the flowers studied shows that, on the whole the 'red' flowers are more acid than the 'magenta', and the 'magenta' are more acid than the 'blue'. However, the Viola with a 'blue' anthocyanin appears to have a petal-pH of 4·4—4·5. The scarlet, puce and purple Anemones form an ascending series of pH values: so do the red, magenta and blue *Primula sinensis*. *Delphinium tatsienense* with a pH of 7·3 and *Ipomoea Leerii* with a pH of 7·8 seem to be the most alkaline.

None of the flower-pigments from flowers examined here have as yet been chemically identified, but the pigments of the following closely related forms have been isolated. *Viola tricolor* contains violanin, stated by WILLSTÄTTER and WEIL (34) to be a rhamnoglucoside of delphinidin. *Delphinium consolida* contains delphinin, a diglucoside of delphinidin [WILLSTÄTTER and MIEG (31)]. *Petunia hybrida* contains petunin, a diglucoside of petunidin or monomethyl delphinidin [WILLSTÄTTER and BURDICK (33)]. *Primula hirsuta* is described as containing hirsutin, or delphinidin trimethylether [KARRER etc. (14)]. Violanin, delphinin and petunin are all referable to delphinidin: the species of *Viola*, *Delphinium* and *Petunia* examined by the writer all have pigments belonging to the 'blue' group. Recent work on anthocyanins shows that in most cases the flower-pigments are not single chemical substances but mixtures. For example, myrtillidin is stated to be a mixture of sugar derivatives of delphinidin and syringidin: althaein to be a mixture ot the monoglucosides of syringidin and delphinidin: ampelopsin to be a mixture of syringidin monoglucoside and delphinidin glucoside with a trace of delphinidin monomethyl ether [KARRER and WIDMER (13)]. In view of these facts one would be very hesitant about suggesting more than a strong affinity between the pigments of one group. Moreover, in view of the stress which has been laid from time to time an purely qualitative colour reactions with alkali as indications of detailed structure of anthocyanin pigments [see ONSLOW (16), WILLSTÄTTER and MALLISON (30), WILLSTÄTTER and NOLAN (32), WILLSTÄTTER, ZECHMEISTER and KINDLER (35), PRATT and ROBINSON (18), WILLSTÄTTER and BURDICK (33), ROBERTSON and ROBINSON (20), KARRER, WIDMER et al. (14)], it would not be out of place to emphasise the necessity for the use of known pH values. Even these crude extracts give some useful information, but of course are only a first approximation. FEAR and NIERENSTEIN (8) have calibrated the colour-changes of a pure anthocyanin in relation to pH. They used natural cyanidin chloride, and compared it with the suggested synthetic cyanidin chloride (3 : 5 : 7 : 3′ : 4′ pentahydroxyflavilium chloride). They state that the natural product was pure blue at pH 8·04, while the synthetic product required a pH of 11·57 to give pure blue. They also indicate that the time-factor plays an important part in the reaction, the colour varying according to the time of observation. This work of FEAR and NIERENSTEIN shows the uncertainty of indentification of particular anthocyanins by uncalibrated reactions with alkali. In the present state of the subject the use of absorption spectra seems to be of little value for identification:

SCHOU (21) has shown that such different substances as paeonidin chloride and malvidin chloride give the same absorption spectra, both of them showing a strong absorption band about 2700 Å. The available data do not enable one to decide whether the 'red' 'magenta' and 'blue' groups represent three different types of anthocyanin, or whether there are only 'red' and 'blue' pigments and 'magenta' is a mixture. (This is the view taken by BUXTON and DARBISHIRE: the writer inclines to the former view.) Until many more pigments have been isolated, purified and calibrated, it will not be possible to give exact chemical meaning to the colour-ranges obtained with these crude extracts, but even grouping the flower-colours according to the BUXTON and DARBISHIRE scheme reveals some interesting points, both of genetic and of systematic importance.

If the flower colours of *Primula* are considered, it is seen that *Primula burmanica* and *P. pulverulenta* both have magenta flowers, and the anthocyanin appears to belong to the 'magenta' group in both cases. The type-form of *Primula japonica* also has magenta-red flowers: there is a salmon-red form of *P. japonica*, described as 'near Briscoei', which has a *red* anthocyanin: that is ,systematically these plants are similar, chemically they are distinct. *P. Cockburniana* has an orange flower, which proves to have yellow plastid pigment, abundance of flavone, and a 'red' anthocyanin. *P. Bulleyana* has a flower of a strong orange yellow: it has plastid pigment and flavone throughout, with a 'red' anthocyanin on the backs of the petals and the tube of the corolla. *P. Beesiana* has a bright magenta flower. A hybrid between *Bulleyana* × *Beesiana* has a bright pink flower, with a yellow eye. It shows the presence of plastid pigment, flavone and a 'red' anthocyanin. Evidently the hybrid has inherited the plastid equipment of the *Bulleyana* parent, and the sap pigment is also of the same type, but much increased in quantity and distribution.

The flower colours which were studied in *Primula sinensis* were full red ('Etna'), deep magenta ('Reading Ruby'), and blue ('Czar'). The genetical behaviour of these colours has been extensively investigated [GREGORY (9); GREGORY, DE WINTON and BATESON (10)], and the factorial composition assigned to red is bR: magenta is BR, and blue, Br. Three possibilities suggest themselves: i) There are three different pigments. ii) There are only two pigments, one redder and one bluer, and the magenta (ruby) is a mixture. iii) There is only one pigment which shows different colours in cell-sap of varying pH. If the third possibility is the correct one, then the extracts from the different flowers should give similar

tints at the same pH values. It was stated by the writer in a previous paper [Smith (25)] that this was the case for the pigments of 'Etna' and 'Reading Ruby': this requires correction. The most careful preparations and repeated comparisons show that the three pigments are distinct. It is possible, however, by using a comparator case to superpose extracts from red and blue flowers without mixing them. When this is done, it appears that, at the same pH values, red extract + blue extract give a very good approximation to the colours of the magenta extracts. It is therefor suggested that the factors R and B represent the development of two anthocyanin pigments of different colours, and that r and b represent the suppression of these pigments, or at least their presence only as colourless precursors. The apparent differences in the pH of the cell-sap may be connected with this: it is possible that the formation of the coloured form only takes place within certain pH limits. (Incidentally, the double recessive 'slaty' (br) can tell us nothing about the pH of the cell-sap in which it occurs, since the anthocyanin in this type is said to be in solid form, and therefor cannot be indicating in the living cell).

The brick-red Cinerarias present an interesting problem. The plants tested were derived from two sources: the 'Feltham Beauty' from Suttons of Reading, and a so-called scarlet 'grandiflora' type from Storrie of Glencarse. Both showed pigment of the 'red' type. The writer then obtained from Storrie a batch of plants of the pink strain from which the scarlets had been selected. These pinks were of varying shades from pale to deep rose-pink, but *all* proved to have '*magenta*' anthocyanins. Considering the high degree of hybridity of the greenhouse Cineraria, it is not surprising that the brick-red strain is by no means fixed. It is, however, surprising to find that it has arisen from a strain with anthocyanin of a different class. If, as seems likely, these anthocyanins are chemically distinct, we have here a hereditary difference of a chemical nature which has apparently arisen in the course of segregation in a highly hybrid strain. The writer is raising a family of strictly selfed brick-red Cinerarias which should give some indication of the diversity of pigments existing in the present strain.

The writer is indebted to Professor W. Wright Smith, Regius Keeper, The Royal Botanic Garden, Edinburgh, for the material of hardy Primulas used in this investigation.

Some of the results, described here in detail, were presented to the British Association for the Advancement of Science at the meeting in London in September 1931, under the same title as the present paper.

SUMMARY

1. A method is presented by which the colour-range of the anthocyanin pigments of any particular flower can be calibrated in relation to hydrogen ion concentration.

2. The grouping of the anthocyanin flower colours as 'red', 'magenta' and 'blue' by BUXTON and DARBISHIRE is confirmed.

3. The necessity of calibrating the pH before accepting qualitative colour reactions with alkali as evidence of chemical structure of anthocyanins is emphasised.

4. It is considered that these natural indicators show colours *in vitro* which are comparable to those shown in the living cell, so that by matching the petal with the pH standards the pH of the cell-sap can be determined.

5. The flowers examined showed pH values from 3·1 to 7·8. The red group was the most acid, and the blue group on the whole the most alkaline.

6. This method allows of direct observation of pH changes accompanying such activities as opening and withering of flowers and the effect of external conditions on them.

7. The relation of petal-pH and pigment type to the genetics of flower-colour in *Primula sinensis* and *Cineraria* is discussed.

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EXPLANATION OF PLATES V AND VI

Plate V. 'Red' and 'blue' anthocyanins

Row *Flower.*

1. Scarlet *Anemone.*
2. Brick-red *Cineraria.*
3. Scarlet *Hibiscus.*
4. *Cineraria* "Wonder Queen."
5. *Delphinium tatsienense.*
6. *Viola cornuta.*

Plate VI. 'Magenta' anthocyanins

1. Puce *Anemone.*
2. Ruby *Cineraria.*
3. *Primula japonica.*
4. *Primula sinensis,* crimson.
5. *Primula sinensis,* ruby.
6. *Primula sinensis,* blue.

PLATE I: 'RED' ANTHOCYANINS

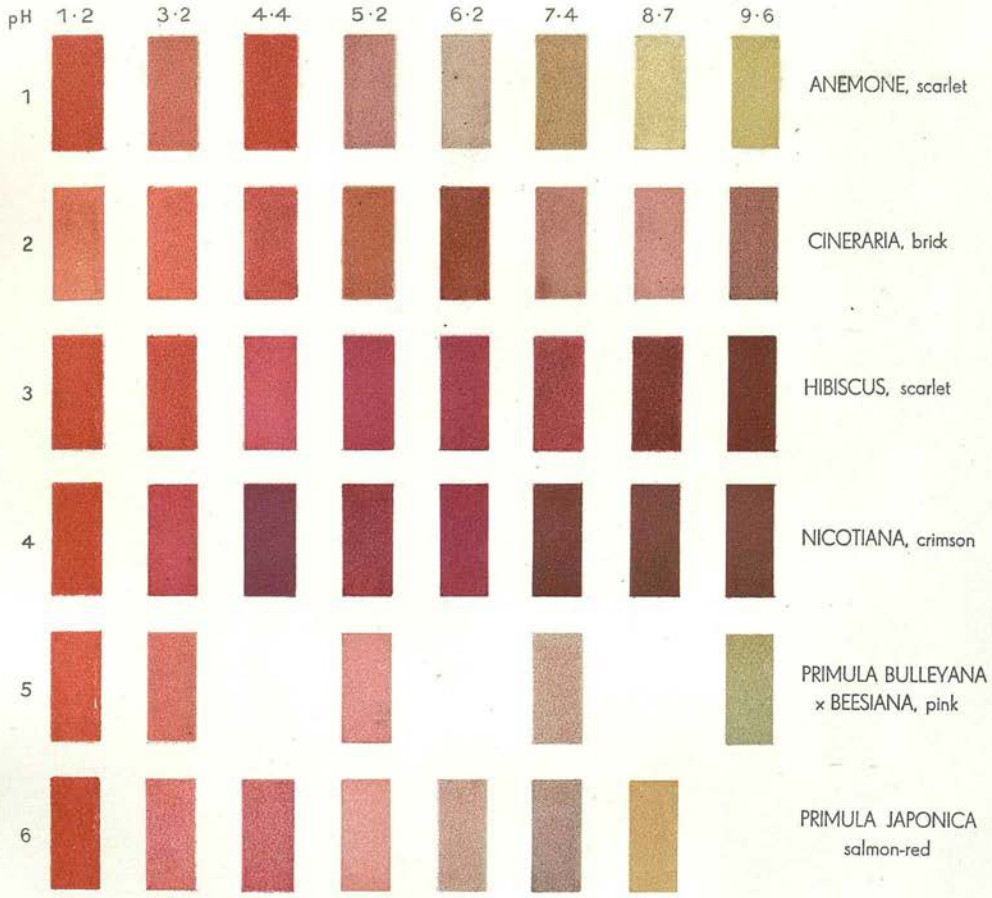

PLATE II: 'MAGENTA' ANTHOCYANINS

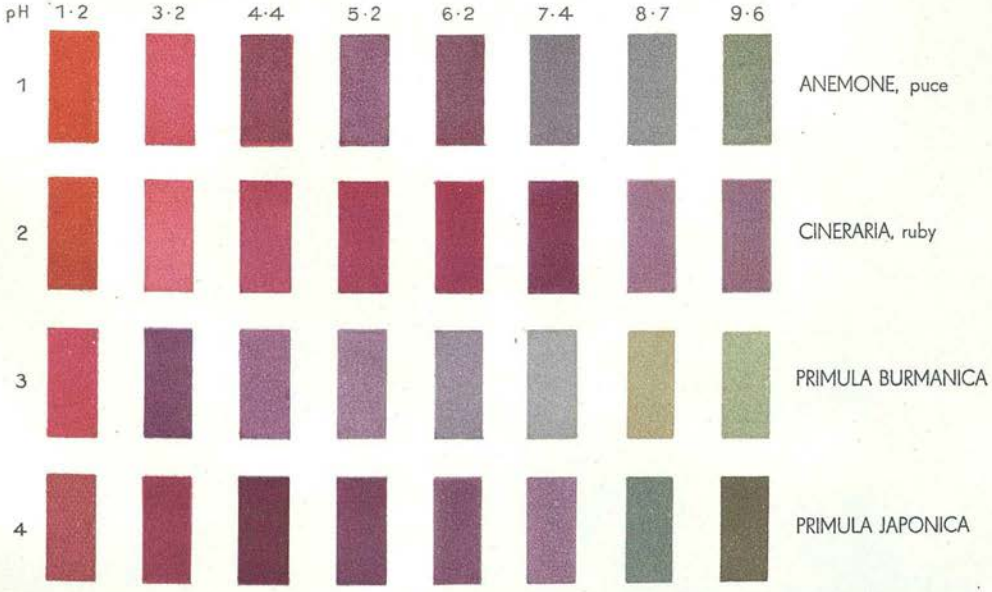

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PLATE II: contd.

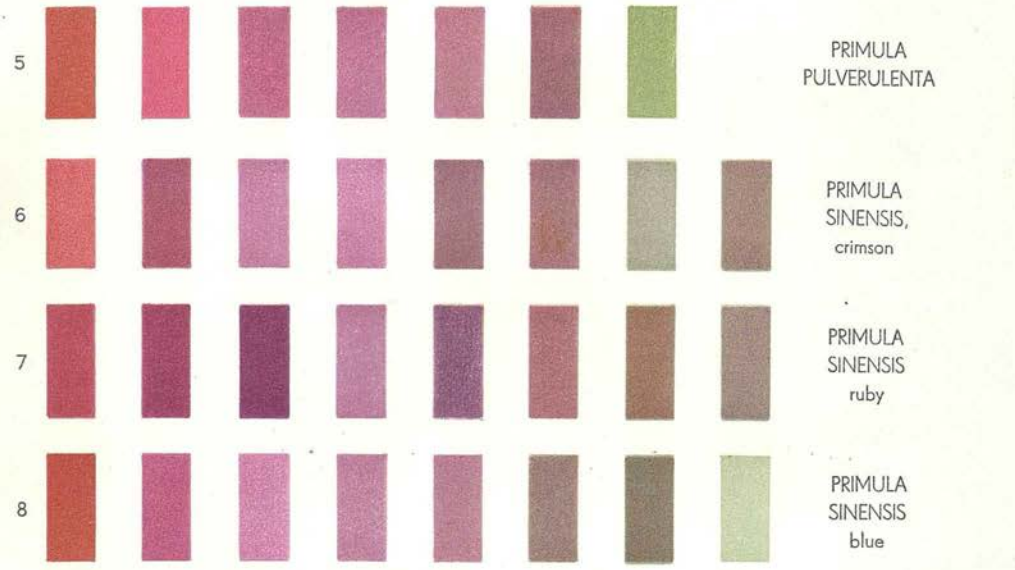
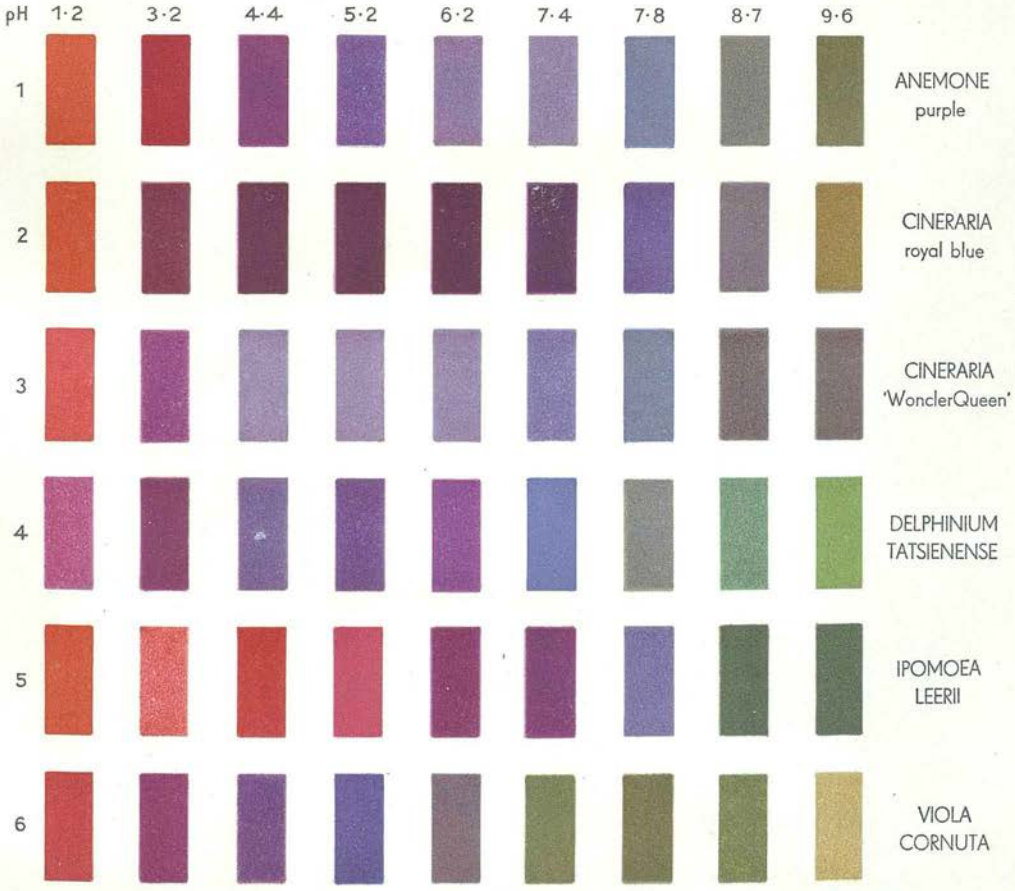


PLATE III: 'BLUE' ANTHOCYANINS



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[Reprinted from "The Scottish Naturalist," November-December 1934.]

THE NATURAL HISTORY OF SOUTH RONA.

THE RESULTS OF A BIOLOGICAL EXPEDITION FROM UNIVERSITY COLLEGE (UNIVERSITY OF ST ANDREWS), DUNDEE, JULY 1933.

Edited by Professor A. D. PEACOCK, Dr EDITH PHILIP SMITH, and C. F. DAVIDSON, B.Sc.

(*Continued from p.* 127.)

III. THE VEGETATION OF SOUTH RONA.

By EDITH PHILIP SMITH.

THE island of Rona lies between Skye and the mainland. It is about 4¼ miles long by 1¼ miles wide at the widest part. The highest point on the island is Meall Acairseid (404 feet). The island lies to the north of Raasay, separated from it by Caol Rona (Kyle Rona), a turbulent stretch of water 1½ miles wide.

The island is composed of worn Lewisian gneiss, and the general aspect is rocky and barren. The general configuration is a series of rocky ridges and furrows, running mainly in a north-west, south-east direction. There are no streams of any importance on the island, and only two bodies of water (see below, also Fig. 3).

The soil is poor and mainly acid. The *p*H values for each type of habitat (obtained by using the British Drug Houses Soil Indicator Apparatus) are given below under each heading.

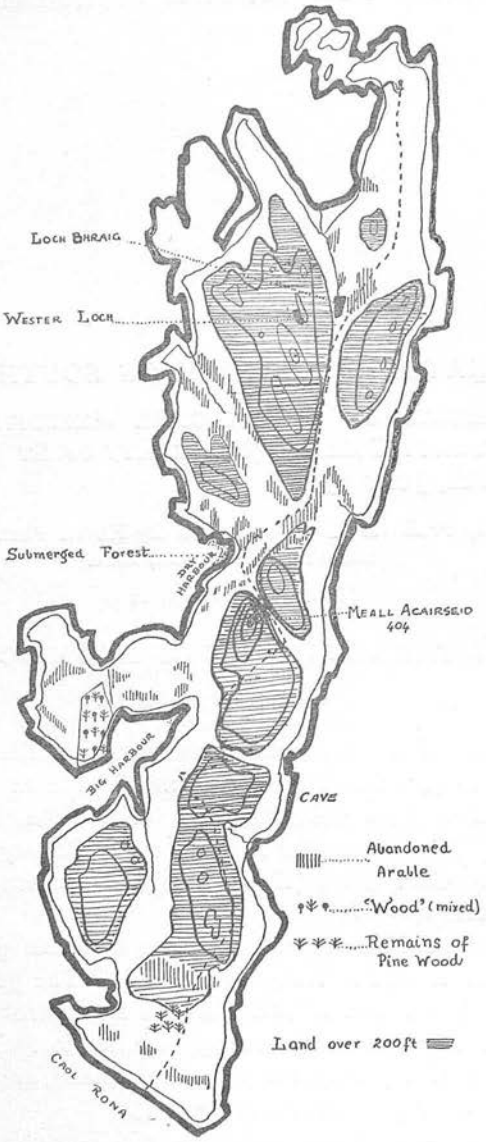


FIG. 3.—SOUTH RONA: scale approximately 1·25 inches to 1 mile.

Rebroduced from the Ordnance Survey Map, with the sanction of the Controller of H.M. Stationery Office. Printed by Oliver and Boyd, Tweeddale Court, Edinburgh 1.

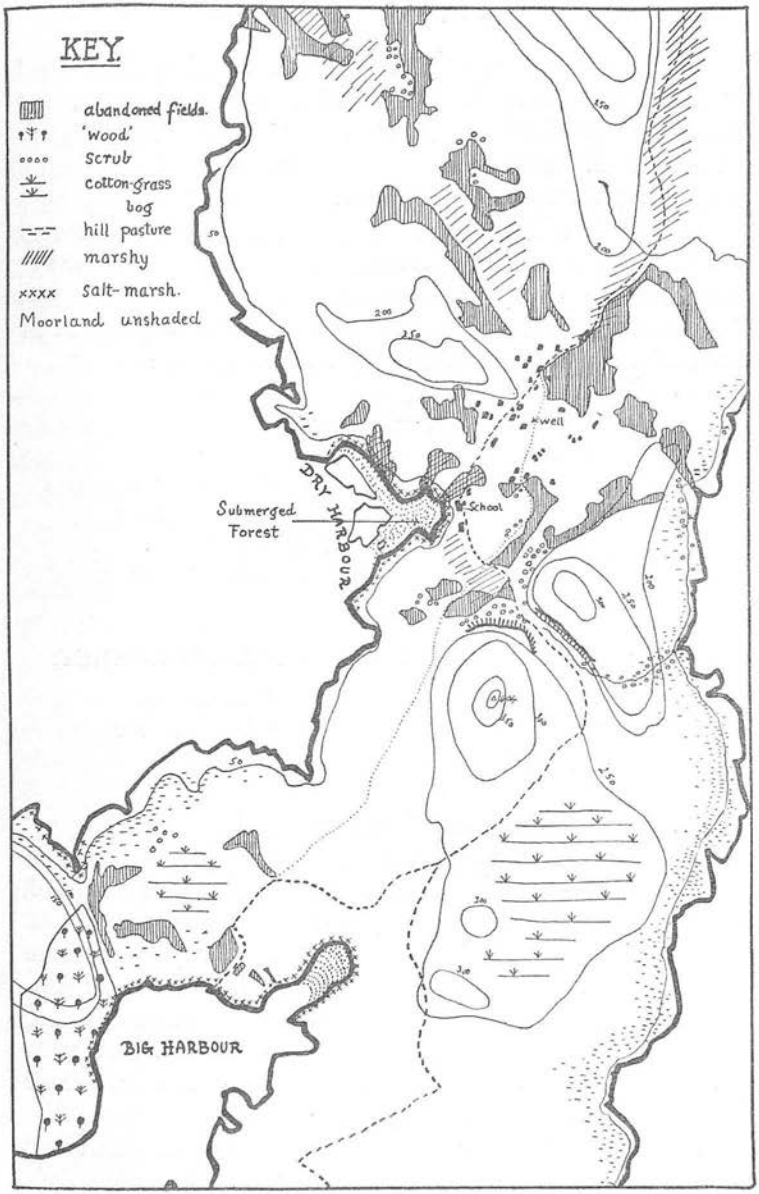


FIG. 4.—Central Part of Island of South Rona: scale approximately 3·5 inches to 1 mile. Outline of fields and path from 6-inch Ordnance Map. Contours added from 1-inch Ordnance Map.

There is one path on the island, running from Caol Rona in the south to the lighthouse in the north, and touching Big Harbour and Dry Harbour (see Map, Fig. 4). It is now impassable for a wheeled vehicle, and sufficiently rough even for the foot-passenger.

On an island as small as this, grazed by sheep and cattle, and formerly supporting a population of 159 (1883), the influence of man penetrates almost everywhere. It is possible, however, to distinguish types of vegetation as natural, and others as affected by human agency:—

A. NATURAL VEGETATION.

(1) Salt marsh
(2) Lowland marsh
(3) Upland marsh
(4) Moor
(5) Peat-bog
(6) Hill pasture
(7) Cave
(8) Fresh-water aquatic
(9) Thalassiophyta
(10) Woodland
(11) Rock community.

B. VEGETATION AFFECTED BY HUMAN AGENCY.

(1) Planted wood
(2) Arable
(3) Roof-top vegetation
(4) Peat cuttings
(5) "Sea-weed Farm"
(6) Various.

The names used are those given in Bentham and Hooker's *British Flora*, 7th edition, 1930.

GENERAL.—Rona, like many another Hebridean island, has been abandoned by its population, but unlike St Kilda, where the cessation of cultivation was abrupt, the tilled ground on Rona has only gradually fallen out of use. Consequently the reversion to natural conditions has reached a later stage than in St Kilda, and at first sight nothing but the ruined crofts and the nettles round their doors remain to tell of human occupancy.

The 6-inch Ordnance Survey map shows the cultivated land as it existed about forty years ago. There were then about 133 acres under cultivation in oats and potatoes: an isolated gooseberry bush and a few stalks of rhubarb beside a croft show the extent of the horticultural efforts. The three crofters at present living on the island are content

with a small patch of potatoes and a few heads of rhubarb: they get their meal and flour from the mainland.

The general configuration of the island, as stated above, shows a series of depressions in the general rocky mass, running for the most part north-west and south-east. The north end of the island is less rocky than the south end. The eastern margin of the island drops sharply away in cliffs, broken by steep gullies where a stream comes down. On the western side are two anchorages—Big Harbour (usable at any state of the tide) and Dry Harbour (usable only at high tide). The schoolhouse was at Dry Harbour. Round these inlets, and at other points where the land slopes more gently to the sea, there is developed a small amount of salt marsh (Plate VII, *B*).

The bottoms of the valleys are very wet, and even in the exceptionally dry summer of 1933 their marshy character was obvious.

A. NATURAL VEGETATION.

(1) SALT MARSH (*p*H 7·25–6·75).

The following plants were found in the salt marsh:—

Arenaria serpyllifolia
Armeria maritima
Aster Tripolium
Atriplex patula (succulence very variable)
Cochlearia officinalis (at upper margin of marsh)
Glaux maritima
Plantago maritima
Sagina maritima
Sagina procumbens
Salicornia herbacea (nearest sea)
Triglochin maritimum
Triglochin palustre.

At the upper margin were found:—

Juncus articulatus
Juncus acutiflorus
Juncus squarrosus
Iris Pseudacorus
Oenanthe crocata
Agrostis palustris
Triodia decumbens.

(2) LOWLAND MARSH (*p*H 6·25–5·75). (Plate VIII.)

Plants found:—

Agrostis palustris
Aira flexuosa
Arenaria serpyllifolia
Achillea Ptarmica
Bellis perennis
Caltha palustris
Carduus arvensis
Cynosurus cristatus
Drosera rotundifolia
Euphrasia officinalis

Epilobium palustre
Festuca ovina, var. *vivipara*
Galium saxatile
Hydrocotyle sp.
Holcus lanatus
Iris Pseudacorus
Juncus acutiflorus
J. articulatus
J. communis
J. lamprocarpus
Lolium perenne
Lychnis flos-cuculi
Myosotis sp.
Narthecium ossifragum
Pedicularis sylvatica
Prunella vulgaris
Potentilla Anserina (towards drier margins)
Ranunculus acris
R. auricomus
Rhinanthus Crista-galli
Rumex Acetosa
R. Acetosella
Salix repens
Senecio aquaticus
Spiræa Ulmaria
Stellaria media
Trifolium pratense
T. repens
Triodia decumbens
Veronica scutellata.

(3) Upland Marsh (pH 5·75 – 5·0).

This is found in the upland, undrained valleys, is characterised by the greater proportion of moorland plants, but grades into the lowland marsh where they meet.

Plants found :—

Agrostis palustris
Aira flexuosa
Arundo Phragmites
Calluna vulgaris
Carex spp.
Cynosurus cristatus
Drosera rotundifolia
Erica cinerea
Erica Tetralix
Eriophorum vaginatum
Epilobium palustre
Habenaria bifolia
Holcus lanatus
Juncus communis
Juncus lamprocarpus
Molinia cærulea
Myrica Gale
Myosotis palustris
Narthecium ossifragum
Orchis latifolia
Pedicularis sylvatica
Polygala vulgaris
Potamogeton natans
Potentilla erecta
Prunella vulgaris
Ranunculus Flammula
Rhinanthus Crista-galli
Rhinchospora alba
Salix repens
Senecio aquaticus
Schœnus nigricans
Scirpus cæspitosus
Sphagnum spp.

(4) Moor.

The principal elevations of the island (above 200 feet) are covered with moor, *Calluna* being dominant. On the cliff-tops, especially on the east side of the island, this moor-

land grades into hill-pasture, which is quite closely cropped by sheep. It also passes over into peat-bog where the soil is deeper and the drainage poor. A large area of peat exists on the south-east flank of Meall Acairseid (404 feet in height), and on the north side of Big Harbour, and also towards the south end of the island. These will be described below.

The heather nowhere reaches any great height, and on the bare hill-tops is very short and wiry. The top of Meall Acairseid may be taken as typical of this kind of vegetation. The hill-top is rounded, with outcrops of worn Lewisian gneiss. Growing actually on the rocks were *Sedum anglicum*, *Plantago maritima*, *Plantago Coronopus*. The vegetation on the flat stretches between the rocks showed the following (analysis of a 6-foot quadrate, lying north-south):—

Calluna vulgaris—dominant
Empetrum nigrum—subdominant
Potentilla erecta—abundant
Erica cinerea—abundant
Galium saxatile
Molinia cœrulea
Agrostis sp.
Juniperus communis (one plant, very dwarf)
Viola sp. (leaves only).

In a sheltered crack between the rocks were found *Polygala vulgaris*, *Hypochæris glabra*, *Blechnum Spicant*: scattered patches of *Vaccinium Vitis-idæa* were also found.

A patch of burnt ground showed the following regeneration:—

Calluna vulgaris	250 plants
Potentilla erecta	54 ,,
Molinia	22 ,,
Agrostis	20 ,,
Pteris aquilina	9 ,,
Carex	4 ,,
	359 ,,

An analysis was made of the vegetation on a line of Tertiary intrusion.

(i) On dry ground:—

Scirpus cæspitosus—co-dominant
Erica Tetralix
Calluna vulgaris—abundant
Molinia cœrulea—frequent
Potentilla erecta—occasional.

(ii) On wet ground :—

Rhinchospora alba—dominant
Molinia cærulea—frequent
Schœnus nigricans—frequent
Narthecium ossifragum—frequent
Scirpus cæspitosus—occasional
Erica Tetralix—occasional
Myrica Gale—occasional
Eriophorum vaginatum — occasional
Drosera longifolia—occasional
Drosera rotundifolia—rare.

(5) Peat-bog.

There did not appear to be any great depth of peat on the island: 3·5 to 4 feet was the greatest depth exposed in a cutting. A cutting towards the south end of the island showed the following stratification (see Plate IV) :—

15 inch	black
6 „	pale reddish
15 „	deep brown-red
12 „	black (sodden)
48 „	

There was evidence at various points on the island that peat had at one time been extensively cut. Now only two areas were in use. The peats were cut about 12″ × 8″ × 3″ and stacked in small heaps. The margins of the cuttings abutted on typical cotton-grass moor, with *Eriophorum vaginatum* dominant. The "floor" of the cutting showed interesting regeneration. *Eriophorum vaginatum* and *Scirpus cæspitosus* were co-dominant at this stage, and were growing in beautifully isolated clumps, with occasional plants of *Schœnus nigricans* and *Rhinchospora alba* interspersed. In the drier parts were *Juncus articulatus*, *Erica Tetralix*, *Pedicularis*; in the wetter, *Sphagnum* with *Drosera*, *Pinguicula*, *Narthecium* and *Myrica Gale*. The *p*H of the peat-bog was about 3·75.

(6) Hill Pasture.

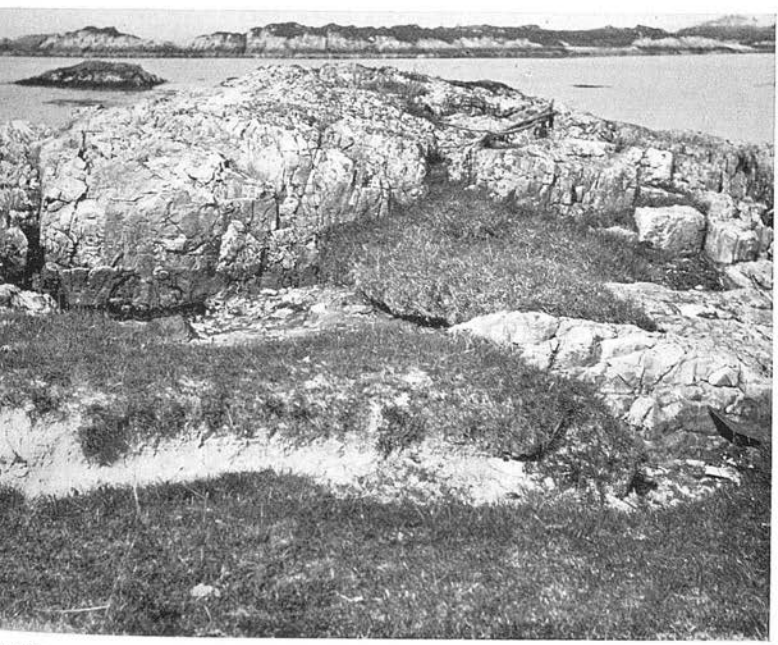
The close-cropped turf extended to the cliff-edge on both sides of the island, often mixed with a good proportion of heather. On the east side, the cliffs are mostly high enough to be out of the reach of salt spray. On the west side they are lower and in some parts wanting. No special alterations in the vegetation in response to spray could be detected.

One of the abandoned fields. Lying in a hollow, running N.-S. Note indication of old field drain in middle of field. A practically pure society of *Juncus communis* with a few wet-meadow plants near drain. Note scrub birch and bracken on opposite slope of ridge.

[*Photo by E. Philip Smith.*

Rocks at Big Harbour showing *Laminaria* zone partially uncovered. Above is *Pelvetia* and *Fucus serratus*. [*Photo by E. Philip Smith.*

1. Planted wood at Big Harbour from the shore. Note untended trees, bent over by prevailing winds. Upper edge of salt-marsh in foreground, with *Juncus*. *Iris*.



2. Salt-marsh vegetation. Turf with *Plantago maritima*, *Triglochin*, etc.

[*Photos by E. Philip Smith.*

Upper end of marsh (an abandoned field) stretching inland from Dry Harbour. It merges into grassy sward and then into moory patches with rocks showing through. Two of the deserted crofts are seen, the one in the centre retaining its thatched roof.

[*Photo by E. Philip Smith.*

The analysis of a typical quadrate of the turf was as follows:—

Calluna vulgaris	100 plants
Ranunculus acris	86 „
Euphrasia officinalis	63 „
Plantago lanceolata	60 „
Rhinanthus Crista-galli	38 „
Molinia cærulea	25 „
Achillea Millefolium	22 „
Poa sp.	18 „
Arenaria serpyllifolia	15 „
Centaurea nigra	13 „
Triodia decumbens	10 „
Trifolium repens	5 „
Prunella vulgaris	4 „
Bellis perennis	3 „
Cerastium vulgatum	3 „
Lolium perenne	2 „
Lotus corniculatus	1 „
Hypochæris radicata	1 „
Rumex Acetosella	1 „
	470 „

A rocky spur projecting into Dry Harbour was covered with moorland vegetation, with bracken and *Vaccinium Myrtillus*. In a crack which ended in a sea-shingle lane were *Matricaria maritima* (succulent) and the Common Dock (only slightly succulent) growing practically in the shingle.

On the eastern side, due east from Meall Acairseid, the cliff-top had been burnt. It was regenerating with seedling *Calluna* and abundant bracken.

Over certain reaches on the west side *Daucus Carota* was common in the turf near the sea margin, and *Sedum Rhodiola* was found on the rocks.

(7) Vegetation of the Cave.

There is a large cave in the cliffs on the eastern side of the island. The mouth of the cave is about 85 feet above sea-level, and the cliff drops sheer to a great depth of water. The cave is roughly triangular in plan, sloping up to the back, where the roof descends and meets the floor. The

floor is covered with a mixture of shell-gravel and peat, well trampled, because this cave was in use as a place of worship at the time of the Disruption. The flora is very scanty. At the mouth, a brake of *Pteris*, with some large plants of *Athyrium filix-fœmina* and *Aspidium spinulosum* screens the opening. Behind that is a huge growth of nettles, somewhat etiolated, with *Galium Aparine*, a Chickweed, *Chrysosplenium* (very pale), and an *Epilobium*. On the walls were *Marchantia*, *Asplenium Trichomanes*, *A. marinum*, *A. Adiantum-nigrum*, *A. Ruta-muraria*, and *Scolopendrium*.

(8) Fresh-water Aquatic.

There are two small lochans on the island. One, Loch Braig, is shown on the Ordnance Map: the other is not, although it is indicated on the Admiralty Chart. The second lochan was mapped and located by this expedition, and is for the present purpose named the Wester Loch. Both are situated in moory hollows, the Wester Loch at a higher elevation than Loch Braig.

Both are characterised by well-developed closed associations of *Arundo Phragmites* round the whole margins. This association is practically pure, only invaded by a few plants of *Scirpus lacustris*, *Sparganium minimum*, and at the outer margin, *Menyanthes trifoliata* and *Ranunculus Flammula*.

The floating-leaved association is pure *Nymphæa alba*, replaced for a few square yards in the centre by *Potamogeton*. Only a small portion of the centre of the loch was open water.

The bottom was muddy and the water drained out slowly by an outlet at the north-west corner. At the edges of this outlet were found *Ceratophyllum demersum*, *Utricularia minor*, *Nitella* sp., while fruiting *Chara* was present in abundance in the outlet stream.

(9) Zonation of Sea-weeds.

The rocks at Big Harbour, Caol Rona, an inlet on the north-east coast, and at Dry Harbour showed similar zonation (Plate VI).

	Occupying about
(i) *Pelvetia canaliculata*	3-4 feet
(ii) *Fucus spiralis* (some *F. vesiculosus*)	5-6 ,,
(iii) *Ascophyllum nodosum* (individual plants 2 feet long)	18-20 ,,
(iv) *Enteromorpha* spp.	6 ,,
(v) *Fucus serratus*, with a little *Ulva*	2-3 ,,
(vi) *Laminaria digitata* (3 feet plants) with much *Leathesia* and some *Furcellaria* on it	15 ,,

Where the rocks go straight down into the sea, the Laminarians are almost entirely replaced by *Himanthalia lorea*, with *Porphyra umbilicalis* growing between. *Chorda filum* grows below the tide-mark. The seaweeds will be referred to again below.

(10) Wood.

Natural woodland is non-existent on the island to-day, although according to the crofters a thick wood, "where the cattle lost themselves," used to surround Loch Braig; this wood is now represented by a few small birches. Scrub birch, isolated *Populus tremula*, *Alnus glutinosa*, *Pyrus aucuparia* (mostly in the gullies), a few willows by the schoolhouse at Dry Harbour, Hazel, Bramble and Ivy indicate that deciduous woodland once occupied a more prominent feature of the landscape.

The remains of a Pine wood, with stumps indicating at least 150-year-old trees, was found towards the south end of the island, but it was impossible to tell by inspection whether this wood was natural or planted, and this was also outside the range of knowledge of the crofters. The ground between the stumps is now peat-bog, partially denuded, and baked hard and cracked when seen in July 1933.

B. VEGETATION AFFECTED BY HUMAN AGENCY.

(1) Woodland.

The almost complete denudation of the woodland on the island is quite typical of the improvident outlook of the earlier crofters. It is true that an attempt was made, some sixty years ago, to replant woodland, and the only wood now standing on the island (on the north side of Big

Harbour) is a result of this attempt. The wood occupies a steep slope facing south-west, and trees are growing right down to the upper edge of the salt-marsh strip. The trees noted were the following: *Betula alba*, *Acer Pseudo-platanus*, *Quercus Robur*, *Fagus sylvatica*, *Cratægus oxyacantha*, *Tilia europea*, *Ulmus montana*, *Fraxinus excelsior*, *Pyrus Aucuparia*, *Larix europea*, *Corylus Avellana*. Undergrowth included Honeysuckle, Ivy, Bramble, with *Poa nemorosa*, *Luzula sylvatica*, *Teucrium Scorodonia*, *Scilla non-scripta*, *Blechnum Spicant*, *Pteris aquilina*, *Galium saxatile*, *Oxalis Acetosella*, *Potentilla erecta*, *Primula acaulis* and *Primula veris*, *Viola* sp., and a good admixture of heather. The trees were very much wind-pruned, especially on the north-east corner of the wood, and none was more than 30 to 35 feet high. Although originally a planted wood, it was obvious that no proper attention had been given it since planting. The trees were unthinned and unpruned, and many showed attacks of *Fomes* (see Plate VII, *A*).

(2) Arable (abandoned).

As indicated above, the arable land on Rona once amounted to about 133 acres (see Map, Fig. 4). Of forty-one fields marked on the Survey map, the writer was able to identify thirty-six: of these, thirty-three had reverted completely to an almost pure society of *Juncus communis*, two had become lowland marsh or wet meadow, and one had gone over to rough wild grasses. Depending upon the elevation, the *Juncus* community was mingled with moorland or meadow plants, but the general appearance was quite characteristic (Plate V). A typical portion showed the following:—

Juncus communis—dominant
Agrostis
Aira
Arrhenatherum—abundant
Festuca
Holcus
Equisetum sylvaticum—frequent
Angelica sylvestris
Cardamine pratensis
Crepis capillaris
Orchis latifolia
Prunella vulgaris
Ranunculus Flammula
R. repens
Scabiosa Succisa
Scutellaria galericulata
Spiræa Ulmaria
Veronica Chamædrys.

One of the more recently abandoned fields near the schoolhouse showed the following :—

Bartsia Odontites
Bromus mollis
Cerastium vulgatum
Gnaphalium sylvaticum
Matricaria inodora
Polygonum aviculare
Potentilla Anserina
Rumex Acetosa
Rumex pratensis
Senecio vulgaris
Senecio jacobœa
Stellaria graminea
Stellaria media
Vicia Cracca.

(3) Thatch.

The roofs of the crofts were thatched with heather, bracken and rush. Few of the crofts at Dry Harbour retained their roofs, and such as were in position were being colonised by *Rumex Acetosa*, followed by *Holcus lanatus* (with a little *Agrostis*). One cottage in particular had almost a full example of moor flora rampant upon it, including *Calluna*, *Erica*, and a large plant of *Athyrium filix-fœmina.*

(4) Peat Cuttings.

These have been described above.

(5) "Sea-weed Farm."

A most peculiar arrangement was discovered at Dry Harbour, and confirmed by reference to the crofters. This can only be described as a "sea-weed farm." The importance of the sea-flora for the inhabitants of Rona will be appreciated when it is remembered that the steep and barren rocks of the island made it impossible to maintain enough cattle to provide fertiliser for the fields. The only source of manure was the "Black weed" (*Fucus*), and the "Brown tangle" (*Ascophyllum nodosum*, and to a lesser extent *Laminaria*). The weeds were raked in, carted up in creels on men's backs, and built up in piles and ridges to decay. In the case of potato cultivation, the weed was drawn into ridges, and when half-rotted the potato tubers were buried in the weed and the whole earthed-up. To encourage the growth of weed in accessible places, stones about the size of a man's head were laid down on the floor

of Dry Harbour. These stones were covered with a rich growth of *Ascophyllum*. In particular, one shallow creek leading out of Dry Harbour had these stones laid in parallel, curved lines running 10 to 12 feet out from the tide-mark. At first it was thought that they were meant for the berthing of the fishing boats or the drying of the nets, but the real purpose is far more interesting, as an example of human ingenuity in the face of difficulties.

(6) VARIOUS.

(a) *Submerged Forest at Dry Harbour.*

Under about 15 inches of sludge well-preserved remains of wood and nut-shells (Hazel) were found. The wood appeared to be Hazel and Alder, but the species are awaiting specialist confirmation.

(b) *Wells.*

The two wells marked on the Survey were located. They were fed by springs, and walled-in with slabs of stone on three sides and above. The one nearest the camp, although within 20 feet of the high tide-mark, was not in the least brackish.

(c) *Tertiary Intrusions.*

Several Tertiary intrusions into the Lewisian gneiss were located. One of the largest, running right across Dry Harbour in a N.W.-S.E. direction, formed at one end a col between Meall Acairseid and another small summit to the north-east. In the gully thus formed the flora is quite luxuriant. The top of the col is typical upland marsh with *Erica Tetralix*, *Agrostis*, *Festuca ovina* var. *vivipara*, *Triodia*, *Molinia*, *Narthecium*, *Carices*, *Eriophorum vaginatum*, etc. A ditch by the path yielded *Aspidium*, *Blechnum*, *Polytrichum*, *Equisetum sylvaticum* and *E. palustre*. The sides of the gully were clothed with Birch, Rowan, Poplar; Ivy, Honeysuckle, Brambles; undergrowth of *Calluna*, *Pteris*, *Athyrium*, *Luzula sylvestris*, *Scilla*, *Oxalis*, *Viola*, etc. The Tertiary

intrusion crossed the floor of Dry Harbour and ended in a clearly defined boss of rock. The line of weakness between this and the gneiss had on one side become a sea-lane with a shingle-covered floor.

There does not appear to be much difference in the flora developed on the gneiss and on the Tertiary intrusions, the only striking point being the apparent dominance of *Calluna* on the one and *Erica Tetralix* on the other.

The following Willows and Sedges were found on the island:—*Salix repens*, various forms; *S. cinerea*, *S. aurita*, *S. aurita* × *repens*, *Blysmus compressus*, *Carex ampullacea*, *C. caespitosa*, *C. distans*, *C. dioica*, *C. flava*, *C. glauca*, *C. Goodenovii*, *C. ovalis*, *C. panicea*, *C. pulicaris*, *C. vulpina*.

The writer is indebted to Professor J. W. Heslop Harrison, F.R.S., Armstrong College, Newcastle-on-Tyne, for naming the Willows, and to Captain John Anthony, M.C., Royal Botanic Garden, Edinburgh, for much help in naming the other plants collected.

(*To be continued.*)

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Nodal Anatomy of Some Common Trees.

By Edith Philip Smith, B.A., Ph.D.

(Read 18th March 1937.)

In presenting these preliminary studies on the nodal anatomy of some common trees four markedly contrasting types have been chosen, namely: *Quercus*, with spiral phyllotaxis and a dissected stele; *Platanus*, with distichous phyllotaxis and a dissected stele; *Acer*, decussate phyllotaxis and a highly dissected stele; *Tilia*, distichous phyllotaxis and a continuous stele. It will be shown that the nodal structure is different in each case, especially in regard to the relative size and persistence of the median and lateral leaf-traces, and the relation of leaf gaps and rays. They will be discussed in detail below.

The material studied was collected in the first week in June. Fixation was in Bles' Fluid (Chamberlain (2)). Serial sections were cut in paraffin at 10 μ, and stained with either Safranin and Anilin Blue in Clove Oil (Chamberlain (2)), or Mallory's Ferric Alum Haematoxylin (McClung (9)). A series of drawings of transverse sections were made with the microprojector, and from these the nodal plans were constructed.

NODAL STRUCTURE OF QUERCUS.

General Structure of the Foliage Twig.

The young leafy shoots of *Quercus* are a warm grey in colour, with a five-angled outline. The leaves, which are practically sessile, are inserted by slightly expanded bases on the ridges of the stem. The ridges are most prominent immediately under the node and gradually decrease downwards.

Phyllotaxis.

The phyllotaxis of the Oak is spiral, and is commonly described as a 2/5 spiral; that is, every sixth leaf is in vertical alignment with another, and the spiral connecting leaf 1 with leaf 6 winds twice round the stem.

Later growth-twists of the internode may obscure the pattern in an older twig, but the situation may be summed

up by saying that five leaves make a pattern, which is indefinitely repeated in the length of the shoot, and which is impressed on the stelar structure of both node and internode.

Vascular Structure. I. *Internodal.*

The stele of *Quercus* is dissected. A transverse section of a young twig shows a five-lobed stelar outline and a five-lobed pith (fig. 1). Taking the largest lobe as 1 and counting clockwise, they are arranged in order of decreasing size as follows: 1, 3, 5, 2, 4. As will be seen below, the stelar lobes consist mainly of median leaf-traces, so that the sizes of the lobes correspond to the proximity of their related leaves.

II. *Nodal.*

The entire primary stele is made up of common segments. Serial sections cut through twelve successive nodes of a young twig of *Quercus* confirm the statement made above that *five leaves* constitute a pattern which is repeated throughout the length of the twig.

In the description below the leaf of origin will be called leaf 1, and the ridge upon which it is inserted will be called ridge 1, the other ridges and corresponding stelar lobes being numbered clockwise. Leaf 6 is the beginning of a new leaf pattern.

The bud is inserted symmetrically on its corresponding ridge. In section it is seen that the bud gap appears in the middle of stelar lobe 1. At the node the whole stem increases in transverse diameter, and the stele also enlarges. The main stele reaches its greatest expansion just before the insertion of the bud-ring. The bud-ring opens on the adaxial side, makes contact with the edges of the gap, and without much contraction opens again on the abaxial side to give the median leaf gap (figs. 1 and 2).

There are three leaf-trace contributions and three corresponding gaps at each node (figs. 1 and 2). This corresponds to the trilacunar types of node described by Sinnott (10) as the commonest and most ancient type of node in the angiosperm. The median trace, which consists of five bundles and is much larger than the lateral traces, enters a wide gap which has opened at the middle of its corresponding stelar

ridge (reinforced as mentioned above by the bud ring) (fig. 1). The median trace runs through two internodes as a whole, then the right lateral third of the trace tapers off against its nearest lateral trace from a higher leaf, while the remaining

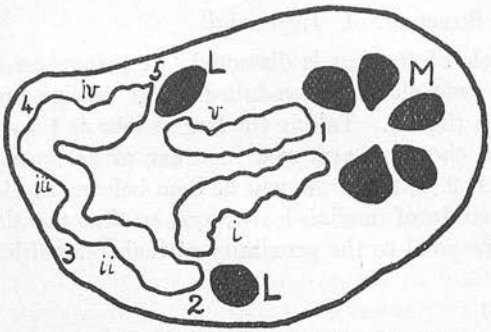

FIG. 1.—T.S. node of *Quercus*, showing entry of leaf-traces. ×20.
M = median trace, composed of five bundles.
L = lateral trace, composed of a single bundle.

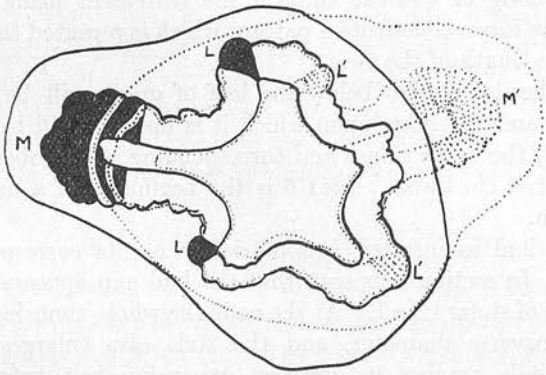

FIG. 2.—Tracing of two successive nodes of *Quercus*, superposed to show the relations of the stelar lobes and furrows with the leaf-traces. The stele is shown at the stage of greatest expansion, immediately after the entry of the leaf traces. M, L = median and lateral traces, leaf 1; M′, L′, same from leaf 2. ×20.

2/3 of the median trace soon splits again. The left third tapers off first, at the level of the third node below the node of origin, while the remaining middle-median portion tapers off to the left of the bud gap immediately below its leaf of origin (leaf 6). That is, the median contribution as a whole persists through *one repeat* of the leaf pattern only (see nodal plan, fig. 3).

The lateral leaf gaps appear at the sides of ridges 2 and 5; that is, the two ridges which are nearest the median trace (figs. 1 and 2). The gaps open towards the bud. Each lateral trace runs straight down through five internodes

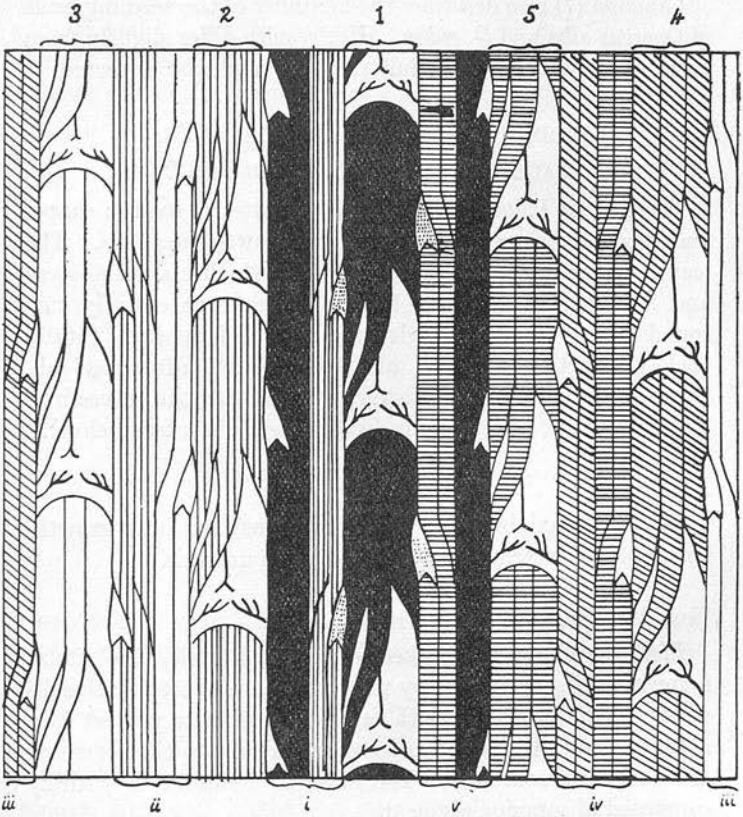

FIG. 3.—Nodal plan of *Quercus* to show the course of the leaf-traces in the stem. The whole stele is shown unfolded in one plane, two complete leaf patterns being included. Ridges (1–5) and furrows (i–v) numbered to correspond with fig. 1. The leaf-traces from the five leaves of a pattern are shaded differently. Vertical scale much reduced.

(one leaf pattern) then moves over towards the bud, thus opening the gap for the entrance of the corresponding lateral from the leaf immediately under its leaf of origin. The lateral traces then run through one more leaf pattern before fading out (see nodal plan, fig. 3).

The node of *Quercus* has been previously described by

Frank (4), with whose results these agree in the main. He describes, however, a branch from the median trace of the leaf vertically above the node of origin as contributing to the bud ring at the latter node. This is incorrect.

Langdon (7) also describes the anatomy of the seedling buds of *Quercus alba* and *Q. rubra*. Her results differ slightly from those obtained from the adult stem, as might be expected.

NODAL STRUCTURE OF PLATANUS.

General Structure of the Foliage Twig.

The twig of *Platanus* is dark green, flattened oval in shape, and generally somewhat twisted as growth proceeds. The leaves are petiolate with large stipules which clasp the stem and may persist after leaf-fall. The leaf-scar entirely surrounds the stem. The buds are small and pointed, totally enclosed in the leaf-base, and only visible after leaf-fall. The petioles are slightly twisted so as to bring the leaves into the same plane, but not so markedly as in *Tilia* (see below).

Phyllotaxis.

The phyllotaxis is two-ranked (distichous), but later growth-twists of the internode may slightly obscure this.

Vascular Structure. I. *Internodal.*

Platanus shows a markedly dissected stele, the stelar segments being separated by rays which are two to three cells wide. There is a large pith, and the segments project into it for varying distances. Sclerenchyma caps to the segments are early differentiated. The stele of *Platanus* is entirely composed of common segments.

II. *Nodal.*

At each node seven leaf-trace contributions enter the stele and seven gaps correspondingly appear (multilacunar type of Sinnott (10)). These may be designated median, M, laterals L1, L2, L3, and laterals L′1, L′2, L′3 (fig. 4). Their subsequent history is as follows.

Median.—The median contribution consists, at the stage when it is approaching the gap, of four distinct bundles (figs. 4 and 5). These enter the ring and then run vertically

downwards through one internode (sometimes splitting and reuniting *en route*). Taking the leaf of origin as leaf 1, at the internode corresponding to leaf 2 the medians move in pairs away from the mid-line and fuse for a time with L3 and L'3 from leaf 2. This results in the opening of the median gap for leaf 3. At the entry of the medians from leaf 3 the composite segment L3–MM splits temporarily, and then, moving still farther away from the mid-line, fuses with and

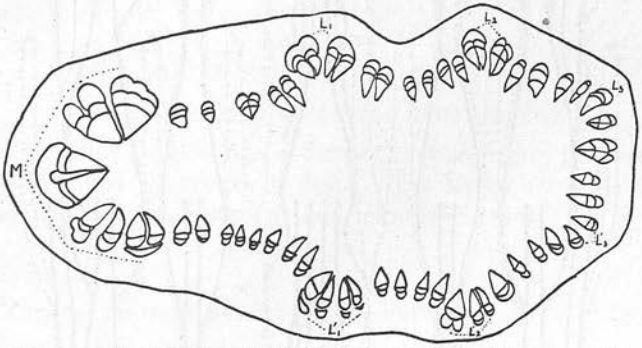

FIG. 4.—T.S. node of *Platanus*, showing entry of leaf-traces. ×20.
M = median trace.
L1, L'1 = first lateral traces.
L2, L'2 = second lateral traces.
L3, L'3 = third lateral traces.

loses its identity in one of the strands of L2 from leaf 1. That is, the median strands pass through two internodes before losing their identity (fig. 5).

Lateral 1.—L1 is composed of two bundles. They pass unaltered through one internode, move apart during the second internode, and finally taper off on the corresponding bundles from leaf 3 (the leaf immediately below the leaf of origin), having temporarily fused with their adjacent laterals L2 and L1 from leaf 2 (fig. 5).

Lateral 2.—L2 is also composed of two bundles. The left bundle of the pair swings out in the second internode to fuse temporarily with the right bundle from L1 of the second leaf, while the right bundle of L2 receives contributions at different levels from the composite trunk L3–MM. After running together with these through the second internode, L2 swings back and fades off on the corresponding L2 of the second leaf, on which the remains of L3 also fades out at a lower level (fig. 9).

Lateral 3.—L3 consists of a single bundle. It fuses with MM during the first internode, runs conjointly with L2 through the second internode, separates from L2 at the third node, and finally fades out on L2 from the third leaf (fig. 5).

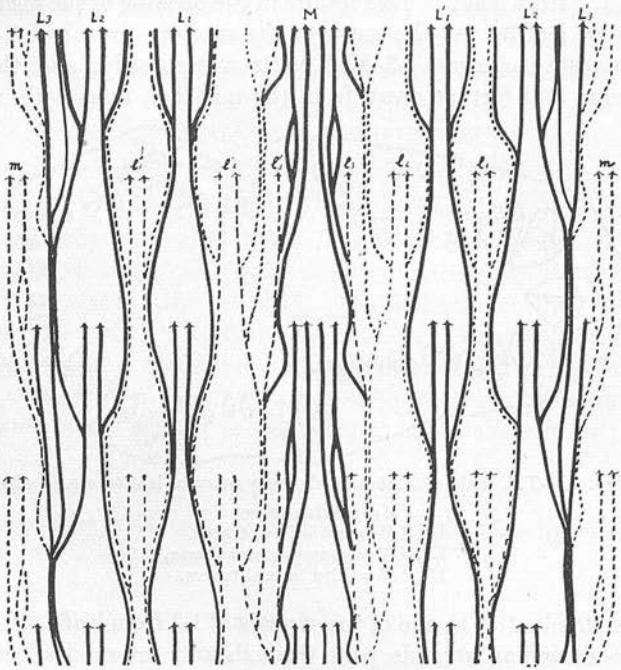

FIG. 5.—Nodal plan of *Platanus*, stele shown unfolded in one plane. Two leaf patterns shown. Traces from successive leaves in solid and broken lines alternately. Lettering as in previous figure (M, L, etc. = traces from leaf 1; *m*, *l*, etc. = traces from leaf 2).

It will be seen that the median traces have the shortest course. They separate and move away from the mid-line, each group, as it were, straddling the gap where the next medians enter immediately below.

The laterals L1 and L2 also move apart to leave the gap for their successors, but swing in to fuse with them after three internodes.

The third lateral slants steadily away from the mid-line, and after three internodes fades out on its adjacent L2, having combined with the median traces just previously.

The bud supply connects with the combined trunk L3–MM.

It will be obvious that the addition of seven leaf-traces, comprising fourteen bundles, at each node will result in a great expansion of the stele at the node, as mentioned above under *Quercus*. The effect of the node is very long-lasting: in fact the one-sided protrusion of pith and stele can still be seen less than 1 mm. above the insertion of the bud; the stele forms in fact an eight-sided figure with one larger curved side, the side towards the insertion of the bud. The pith shape is similar.

NODAL STRUCTURE OF ACER.

General Structure of the Foliage Twig.

The young twigs of *Acer* are smooth surfaced, grey in colour, and rounded hexagonal in shape. The leaves are petiolate and attached to the stem by large crescentic bases.

Phyllotaxis.

The leaf arrangement is opposite and decussate.

Vascular Structure. I. *Internodal.*

The young twig of *Acer* shows a definitely dissected stele, with twenty-four wedge-shaped bundles, widely spaced, and a large pith. The general outline of the stele in section is hexagonal, and it has two axes of symmetry, the longer lying between the angles of the stele corresponding to the nearest node, the shorter at right angles to it (figs. 6 and 7). The alternation at right angles of the successive pairs of leaves leads to a reversal of the position of the longer and shorter axes in each successive internode.

II. *Nodal.*

As there are two opposite buds and leaves at each node, which behave alike, one-half of the stem only will be described.

There are three leaf-trace contributions and three corresponding gaps (trilacunar node) in each half of the stem. Of the three traces the two laterals appear considerably earlier than the median—in fact they are to be seen running horizontally in the cortex even before the bud contribution has amalgamated with the stelar ring (fig. 6).

The median trace consists of three bundles—one large central

bundle flanked by two smaller ones. These three bundles come into the stem together, and together run down vertically through one internode. At the next node (node 3, immediately below the leaf of origin) the large central bundle splits and moves apart, and the two smaller strands meet and taper off on its flanks about half-way down the second internode. At the third node (that is, the node immediately under the leaf of origin) the two halves of the median strand separate

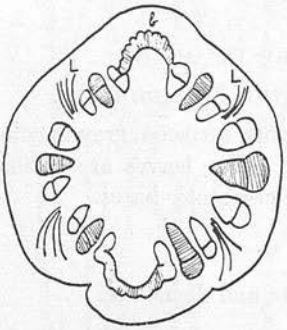

FIG. 6.

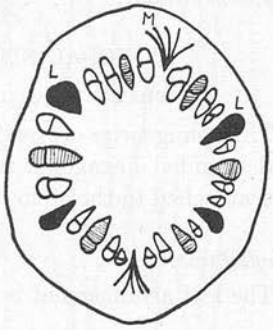

FIG. 7.

FIG. 6.—T.S. node of *Acer*, showing entry of bud-ring (*b*) and lateral leaf-traces (L). ×20.

FIG. 7.—T.S. node of *Acer*, later stage, showing entry of median leaf traces (M). The lateral traces are well within the main stelar ring (L). ×20.

still more to form the bud and leaf gaps for that node, and then curve in again in the course of the third internode, to fade out on the smaller median strands from the third node. It will be seen that the mid-median strand in each case splits and straddles its immediate successors—the whole median trace of the node immediately below it (see nodal plan, fig. 8).

The lateral traces enter the stelar ring before the median: they are composed of single bundles. They run straight downwards through one internode. In the second internode they move over towards the bud, then run vertically through a third internode before tapering off on the corresponding laterals from leaf 2. It will be seen that the mid-median and lateral traces keep their identity for almost the same distance; the laterals for slightly longer than the median (figs. 6, 7, 8).

The bud contribution is received on the inner aspects of

the halved mid-median traces from the node immediately above the bud (fig. 6).

The duplication of the triple leaf-trace contribution at each node leads to the hexagonal shape of pith and stele referred

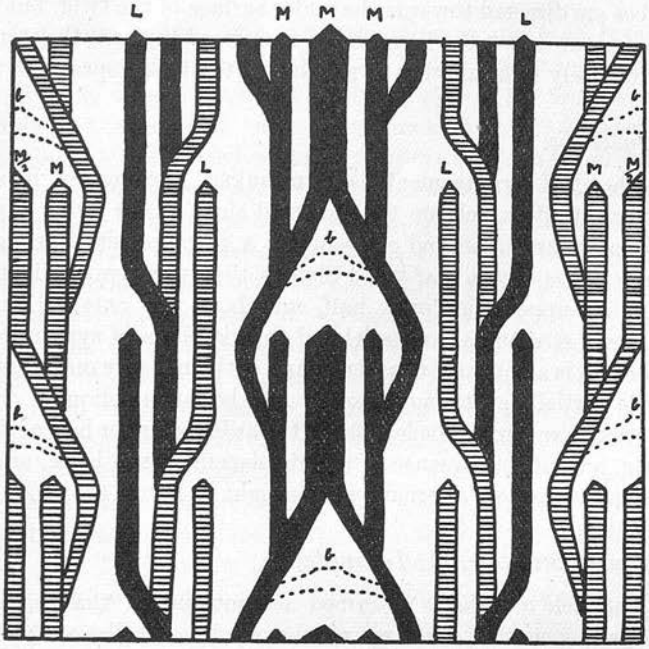

FIG. 8.—Nodal plan of *Acer*, showing stele unfolded in one plane. Vertical scale much reduced. The traces from leaves in the same vertical rank are shaded alike. M = median traces; L = lateral traces; *b* = bud-ring.

to above. The pith shape and the position of the primary segments can be seen in older branches owing to the persistence of the primary rays, even when secondary cambial activity has given the wood-mass a circular outline externally (compare with *Platanus*, *Tilia*, *Quercus*).

NODAL STRUCTURE OF TILIA.

GENERAL STRUCTURE OF THE FOLIAGE TWIG.

The general appearance of the spray of *Tilia* is flat, because the leaves are arranged alternately on either side of the twig, and their petioles are twisted so that the blades lie in one

plane. The stem is rounded in outline, slightly flattened from side to side, and is not straight but zigzag throughout its length. The foliage leaf is slightly asymmetrical, one lobe being larger than the other; in the young spray the larger lobes are directed towards the under surface of the twig, but in the mature spray the larger lobes are turned towards the stem, alternately right and left in relation to the whole spray.

Phyllotaxis.

The leaf arrangement is two-ranked, the leaves being attached alternately on the flattened sides of the twig. The leaf-scars are small and crescentic. A plane passing through the centres of the leaf-bases divides the twig symmetrically into an upper and lower half, and both the external and internal structures can be related to this plane of symmetry. The bud is a flattened oval structure, with one large outer bud-scale partially enclosing the rest. The buds are obliquely displaced (in regard to the leaf-scars) towards the upper half of the twig, and in consequence of this displacement the large outer bud-scale appears alternately to lie right or left of the twig.

Vascular Structure. I. *Internodal.*

The stele of *Tilia* is described as continuous; that is, the stelar ring consists of a large number of close-set files of xylem separated by narrow strips of parenchyma. Even here, however, it is possible to distinguish in the very young stem that certain segments of the stele are delimited by wider strips of parenchyma. A study of the node reveals that these are, in fact, the last remnants of the leaf and bud gaps; I propose to call them *gap-residues*. They persist for a varying length of time and finally merge into narrow rays.

A study of the development of the stele from the procambial stage is in progress. The results are as yet incomplete, but it may be said here that some at least of the protoxylem in *Tilia* is primary (that is, developed from procambium). The relations of the rays, gap-residues and intra-segmental files of parenchyma are being analysed.

The outer margin of the wood is approximately circular in outline, but the pith forms an irregularly seven-sided figure, with a protrusion towards the place of insertion of the nearest

bud. The stelar ring is therefore narrowest at this region. The protoxylem forms a series of fine points which do not protrude deeply into the pith.

II. *Nodal.*

Approaching the node the stele enlarges and then breaks to form the bud gap, the direction of the protrusion of the stele being directed towards the upper side of the twig, at an angle to the mid-leaf plane of symmetry referred to above. The bud stele approaches the gap, opens on the adaxial side, and approaches the main stele at the edges of the gap. The whole stele contracts a little, but not quite to the internodal size, and then the leaf gaps appear, the bud stele breaking on its abaxial side to give the median leaf gap (fig. 9).

The leaf-trace consists of a median and two lateral contributions (trilacunar type): as their times of entry and courses in the stem are somewhat different, they will be described separately as the median and the upper and lower lateral traces respectively, corresponding to the side of the twig to which they pass.

The slightly oblique setting of the leaf-base in relation to the bud leads to an inequality in the track of the leaf-traces (fig. 9). The median trace comes in and moves counter-clockwise, more or less horizontally, until it is opposite the protrusion of the bud stele at its junction with the main stele, where its gap appears; it then slants in and takes its place in the median gap, which is the last of the three leaf gaps to appear.

In the case of the lateral traces, the gap for the upper lateral appears first, then the lower lateral gap. The upper lateral trace moves counter-clockwise in a more or less horizontal direction round the stem; its track is longer than that of the lower lateral because it has to circle round the bud-stele protrusion. The lower lateral trace moves round clockwise till it is opposite its gap. The two lateral gaps appear directly opposite each other, and a line joining them at their first inception crosses the mid-leaf line at right angles. By comparison of successive serial sections it is seen that the upper lateral gap opens *away* from the mid-leaf line while the lower lateral gap opens *towards* it (figs. 9, 10).

The stele is now at its most expanded stage. The leaf-

traces are still more or less horizontal but are approaching their gaps. The traces enter the gaps in the order of their appearance, and the two lateral traces are always well within the ring before the median. The median trace at this stage is seen to be triple, but the segments unite before it joins the

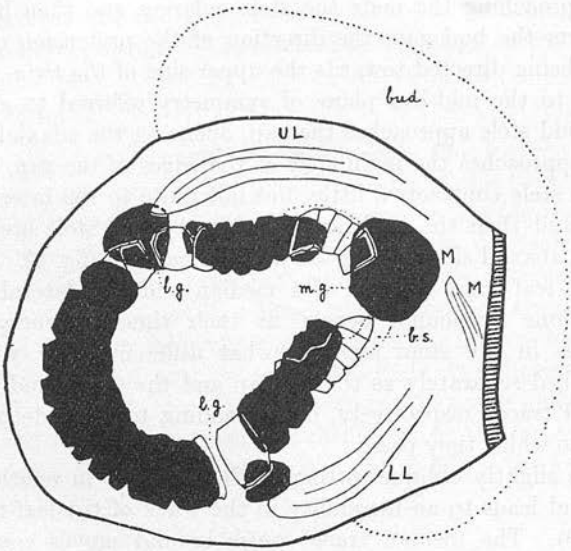

FIG. 9.—T.S. node of *Tilia*, showing stages in the entry of bud- and leaf-traces. ×25.

(i) The bud is shown displaced towards the upper side of the twig; the bud-ring (*b.s.*) is drawn with a dotted line.
(ii) First appearance of the leaf gaps: *m.g.* = median gap; *l.g.* = lateral gap. All three traces (drawn with a fine line) are seen running more or less horizontally in the stem, the median and upper later traces moving counter-clockwise towards their gaps, the lower later moving clockwise towards its gap.
(iii) The leaf-traces are just entering the main stele (solid black), the gaps being open to their widest extent.

stele of the branch. All three traces penetrate the ring deeply, being seen for a short distance distinctly protruding into the pith.

In following the course of the leaf-traces after they have joined the stele series of transverse sections were cut through six or seven successive nodes. In the case of a continuous stele the close setting of the segments makes it difficult to follow out the vertical courses of the traces by this method alone, so that recourse was had to injection methods by which

coloured solutions were forced into the petiole. It was found that the dye had been forced back in the leaf-trace segments for about six nodes below the insertion of the experimental leaf. By a combination of these two methods the course of the traces was determined as follows.

The median segment pursues a straight vertical course through two internodes, and then fades out at the bud gap

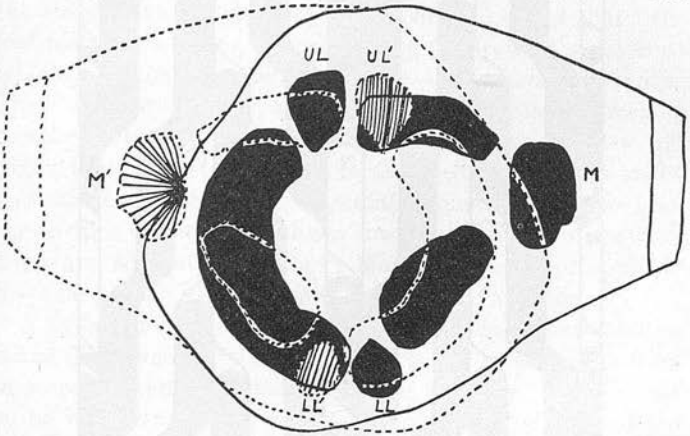

FIG. 10.—Tracings of two successive nodes of *Tilia*, superposed to show the symmetry of the system. ×25.

M, UL, LL = median, upper and lower laterals of node 1.
M', UL', LL' = median, upper and lower laterals of node 2.

Note that a line joining the median traces divides the stem into an upper and lower half, while a line joining the beginnings of the lateral gaps is at right angles to this. The upper lateral gap opens away from the bud, the lower lateral gap opens towards the bud.

of the bud immediately below the leaf of origin, the trace tapering off to the left of the mid-line of the bud. (Taking the leaf of origin as leaf 1, the median trace fades out at leaf 3.) (See nodal plan, fig. 11.)

The lateral leaf-traces pursue a longer course. The laterals from successive leaves come in to right and left of the transverse diameter of the section alternately. The laterals extend vertically through six internodes, but not in a straight line, because they move over at alternate nodes to accommodate the corresponding laterals from the successive leaves immediately below their leaves of origin (fig. 11). Both upper and lower lateral traces move counter-clockwise round the stem in their sinuous course; it will be seen that they each

approach a median segment and finally taper out at the bud gap of the third leaf under their leaf of origin. (If the leaf of origin is called leaf 1, the laterals fade out at leaf 7.)

It will also be seen that the major part of the stele is composed of lateral leaf-trace segments.

FIG. 11.—Nodal plan of *Tilia*, showing the stele unfolded in one plane. Vertical scale much reduced. Two leaves constitute a pattern. Alternate leaf patterns black and white.

M, UL, LL = median, upper and lower laterals of leaf 1.
M′, UL′, LL′ = median, upper and lower laterals of leaf 2.
B = bud supply.

DISCUSSION.

The three types described which show a dissected stele in the young stem (*Quercus*, *Platanus* and *Acer*) all maintain this character in the older stem: persistent wide rays continue to cleave the woody mass, and as long as the pith is intact the original structure of the young stem can be clearly discerned by examination of the pith border. In all these cases numerous secondary rays of minor duration and importance appear (see Bailey, I. W. (1)).

A study of the procambial stage in all four types confirms Kostychev's statement (6) that a continuous procambium ring is the most common case in Dicotyledons—the dissected stele being arrived at by "parenchymatisation" of segments alternating with the vascular segments. A detailed study of this is in progress, with a view to a comparison of the development of the continuous and dissected type of stele in the early stages of growth. Kostychev (6) states that "the leaf-traces in a young wood-bast ring (continuous stele) must be sharply distinguished from the true vascular bundles. They are distinct morphological elements, resulting from a correlation between leaf and stem development." This would imply that in a stem like *Tilia* the "leaf-traces" are to be distinguished from the "true vascular bundles." It has been shown that the stele of *Tilia* is entirely composed of common segments, so that Kostychev's statement does not apply to this case. This point seems to need further analysis.

A feature which has emerged in these preliminary studies is the dominance in the young axis of the structural pattern imposed by the phyllotaxis. This is not new, but can bear to be reaffirmed. The striking studies by Louis (8) of the development of procambium from promeristem in the apex of *Syringa*, etc., re-emphasise this. He shows that the procambial segments destined to become vascular segments differentiate simultaneously in the leaf primordium and in the "soubassement foliaire," and that the full vascular pattern is not established until a certain number of nodes (depending upon the phyllotaxis) have developed. The statement of Langdon (7) that the procambial strands in *Quercus rubra* and *Q. alba* initiates in the leaf primordia and develop both basipetally and acropetally needs re-examination in the light of this newer work.

The phylogeny of the angiosperm stele, about which much has been written (see, for example, Jeffrey (5), Eames (3), Sinnott and Bailey (11, 12)) cannot profitably be considered until much more is known of its ontogeny in the adult shoot.

Particularly is this the case in an attempt at a comparison between the continuous and dissected type of stele (see Eames (3), Kostychev (6), Louis (8)).

It is obvious that much work is still needed on the primary vascular system of the angiosperms.

SUMMARY.

1. In the four types studied the whole stele was composed of common segments.

2. *Quercus*, *Acer* and *Tilia* correspond to Sinnott's trilacunar type, *Platanus* to his multilacunar type.

3. In all four types the median leaf-trace contribution and the bud contribution persisted through one leaf pattern only, making way for the corresponding contributions from the leaf immediately below the leaf of origin. The number of internodes involved in one leaf pattern varies with the phyllotaxis, being two in *Platanus*, *Acer* and *Tilia*, and five in *Quercus*.

4. The median leaf-trace at the time of its amalgamation with the main stele consists in *Tilia* of one segment, in *Acer* of three segments, in *Platanus* of four and in *Quercus* of five segments.

5. The lateral leaf-trace contributions in all cases persist for a considerably longer time than the median, enduring for several leaf patterns before fading out.

6. Just before the entry of the leaf traces the main stele shows a considerable transverse expansion and the gaps are evident as wide bands of parenchyma. Even after the leaf-traces have entered the stelar ring and it has contracted to its internodal size, the new segments are delimited by gap-residues, which persist for at least one internode before merging into rays.

7. In *Quercus* and *Acer* the persistent wide rays in the older stem are also related to the gap-residues.

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